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The Effects of a Water-Soluble Carcinogen on Early Frog Development*

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Since the discovery of the chemical carcinogens a large amount of work has been done with these substances. The emphasis so far has been on the investigation of new chemicals for carcinogenic properties and the correlation of carcinogenicity with chemical structure, while relatively little has been done by way of analyzing the mode of action. The approach to the latter problem depends somewhat on the investigator's concept of the main differences between normal and malignant cells. In the first place, since malignant tumors generally grow faster than homologous normal tissues it is natural to think that carcinogens might have a growth-stimulating effect. The evidence on this point is somewhat contradictory, but the balance of it now appears to indicate that the effect of carcinogens on growth rate is not the primary one involved in the transformation of a normal cell into a malignant one.

A second general difference between normal and malignant tissues is expressed in the statement frequently made that malignant cells are less differentiated than normal ones. According to this concept there is an antagonism between growth and differentiation; normal cells ordinarily cease to divide as they differentiate, while in malignant cells growth is emphasized and differentiation inhibited. If this is a genuine difference, carcinogens might be expected to act in part by inducing dedifferentiation or by impeding differentiation.

Finally there is the hypothesis that carcinogenesis involves a fundamental alteration or mutation which endows the cell with new properties, one of which is the capacity for continued anaplastic growth. According to this theory a carcinogen should act by producing an irreversible heritable change in the cell. It would not necessarily produce primary changes in growth and differentiation, these being altered only after carcinogenesis has been brought about.

In an attempt to settle some of these questions concerning the nature of carcinogenesis, we have exposed developing amphibian embryos to carcinogenic chemicals. Normal development involves both growth processes and processes bringing about differentiation, and these are ordinarily quite exactly integrated to produce a well ordered whole. If carcinogenic chemicals have a primary effect on growth this should be evident from determinations of cleavage rates and growth rates. If there is an effect on differentiation this should be expressed as an alteration of differentiation relative to growth, and possibly as a disorganization or disturbance in the normal relationship of parts in the developing embryo.

MATERIALS AND METHODS

Rana pipiens adults were obtained from the northern Lake Champlain region and kept in the laboratory in slate aquarium tanks in running water at 3-5° C. In preparation for an experiment a female frog was transferred from the stock tank to room temperature (20-23° C.) for one day, following which ovulation was induced by pituitary injection. One to 2 days later the eggs (now in the uterus) were stripped into a sperm suspension made up in 10 per cent Ringer's solution. After the gelatinous layers surrounding the eggs had swollen (about 30 minutes) the individual eggs in the egg mass were cut apart from one another and examined under the dissecting microscope in order to verify that they were normal. They were then placed in a single finger bowl and stirred, so that a perfectly random sample would be distributed to the various test solutions.

One hour after insemination lots of 40 eggs were placed in covered finger bowls, each containing 100 cc. of solution. The finger bowls were then placed in a

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water bath thermostat at 19.6° C. Control animals developed throughout in 10 per cent Ringer's solution; experimental ones in solutions of the carcinogen or other substances made up in 10 per cent Ringer's solution.

The first, second, and third cleavages were timed with a stop watch. Following cleavage, developmental rate was determined by measuring the time required to reach definite morphological stages, identified according to Shumway (32), such as beginning of gastrulation, closure of blastopore, and neural fold fusion. Subsequent to neurulation we observed development of reflexes and rate of increase in total length, as well as morphological development. Reflexes were tested according to criteria used by DuShane and Hutchinson (12). Length measurements were made by means of a graduated stage which moved the embryo across the field of a stationary microscope containing a cross-hair eyepiece. The stage moved on a screw of 1 mm. pitch, the head of the screw being accurately graduated in hundredths, allowing readings to 0.01 mm. The instrument was calibrated with a stage micrometer giving an error of 0.2 per cent for a length of 2.0 mm. During measurements the animals were placed on velvet to prevent movement, and when necessary they were anesthetized with MS-222, *m*-aminobenzoic acid-ethyl ester, in the form of a methansulfonate (30).

In the majority of our experiments, the developing eggs and embryos were exposed to Na-1,2,5,6-dibenzanthracene-9,10-endo-*α*,*β*-succinate (*trans*).[1]

Dibenzanthracene (DBA) was obtained from the Eastman Kodak Company. The succinic acid derivative was prepared by Mr. R. Schissler, of the Department of Chemistry, McGill University, according to the methods of Bachmann and Kloetzel (1) and Warren (37), and had a melting point of 255-256° C. (corrected). The sodium salt was prepared by heating the desired amount of the acid with a calculated amount of 1/10 N NaOH and distilled H_2O (slightly in excess of the amount needed on the basis of 0.2 per cent solubility of the carcinogen). After cooling, the solution was filtered and diluted with 10 per cent Ringer's solution as required. The pH of all concentrations used was checked and found to be the same as that of 10 per cent Ringer's solution.

It was found that the aging of solutions at room temperature, up to 7 weeks at least, had no effect on their activity, although keeping them for the same length of time in the ice box (4° C.) caused precipitation of the two stronger concentrations used, with a consequent reduction of potency by approximately 50 per cent.

Solutions which had been used in one experiment and subsequently filtered were found to be capable of acting again with equal potency on a second group of eggs. This indicated that the effective concentration was not altered during the course of a given experiment.

The concentrations used in our experiments were 0.02, 0.213, 2.13, 4.26, and 10.0 mgm. DBAS per 100 cc. of solution. This range was selected to include values which have been reported to stimulate (0.213) and retard (2.13) the growth of mouse fibroblasts in culture (9, 10).

It may be noted here that Lettinga (20) found that as small a dose as 0.0125 mgm. of DBA could produce tumors in mice (4 of 20 animals), and 0.5 mgm. appeared to be a limiting amount beyond which there was neither decrease of latent period nor increase of tumor incidence. Dobrovolskaia-Zavadskaia (11) reported a single injection of 0.0025 mgm. of DBA to be capable of producing tumors in mice. Levine and Bergmann (21) induced tumors in mice by a single injection containing 0.0033 mgm. of DBA, and observed that repeated injections caused greater injury but no greater tumor production.

By calculating the weight of a mouse at approximately 35 gm., and the water volume at approximately 85 per cent, the effective concentrations of the minimum doses above are found to be 0.04 (Lettinga) and 0.008 mgm. per 100 cc. (Dobrovolskaia-Zavadskaia); and the maximum effective doses are 1.66 (Lettinga) and 0.011 mgm. per 100 cc. (Levine).

Many workers have, of course, induced tumors with much larger doses of DBA, but these calculations serve to show—to the extent that work with mice and Amphibia can be compared—that not only the upper members of our series but the entire range of concentrations might be considered to be roughly within carcinogenically effective limits.

RESULTS

I. The Effects of Continuous Exposure to Na-1,2,5,6,-Dibenzanthracene-9,10-Endo-*α*,*β*-Succinate (trans) on the Early Development of the Frog (12 Experiments; 2,420 Eggs)

Cleavage rates for the first three cleavages were determined carefully and found not to be affected by the carcinogen in the concentrations used. Fig. 1 illustrates the results obtained for second cleavage in a typical experiment. Similarly, development through blastula formation to initiation of gastrulation was unaffected. Eggs exposed to the carcinogen continuously from one hour post-fertilization always developed the dorsal lip of the blastopore at the same time as did the controls (Fig. 2). As gastrulation progressed the first effects of the carcinogen became evident. In the

[1] Henceforth referred to as DBAS.

stronger concentrations (10.0, 4.26, and 2.13 mgm. per 100 cc.) there was a retardation which was roughly proportional to the concentration (Fig. 3). Embryos

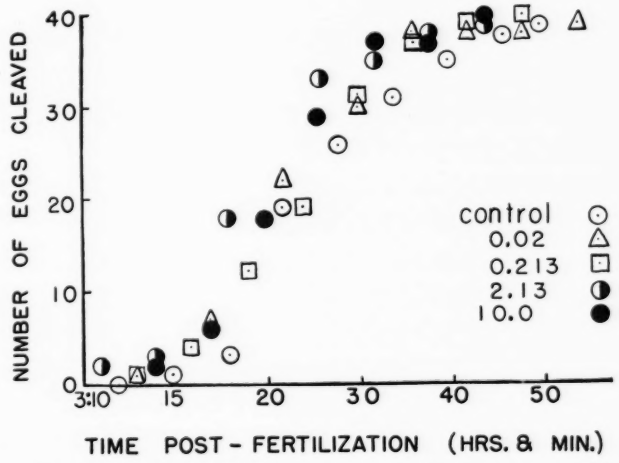

FIG. 1.—Effect of DBAS on cleavage in the frog's egg. This graph is of second cleavage, but is typical of the results observed in the first three cleavages of all experiments.

Concentrations in all figures refer to mgm. per 100 cc.

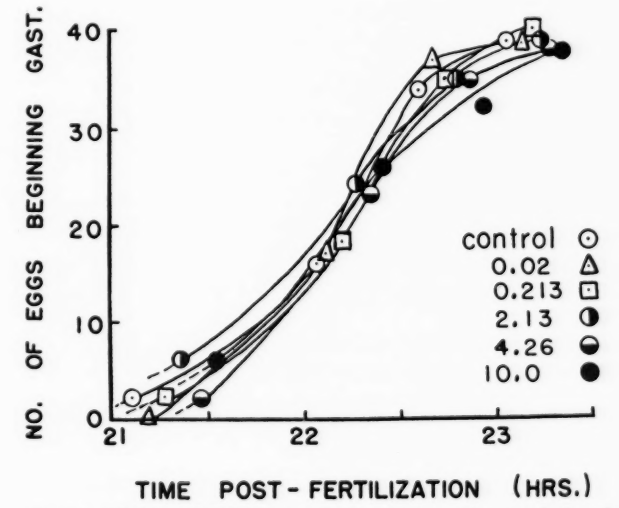

FIG. 2.—Effect of DBAS on the beginning of gastrulation in the frog's egg.

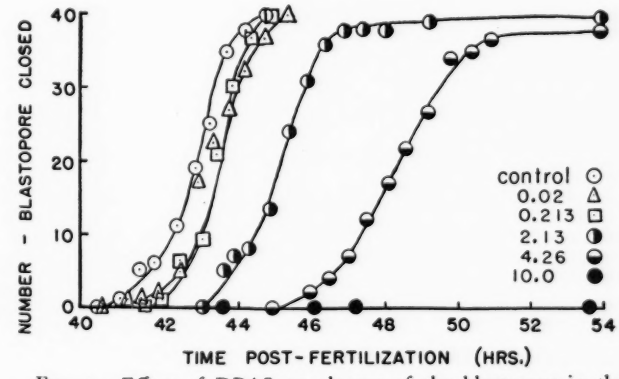

FIG. 3.—Effect of DBAS on closure of the blastopore in the frog's egg.

in concentration 10.0 suffered the greatest retardation; a circular blastopore formed at a slow rate but never closed. Before closure could take place the lips of the blastopore began to degenerate. This degeneration involved first a recession of pigment from the surface of the individual cells, rendering them a pale grayish color in place of the usual dark brown. Following this the cells became swollen and round and finally broke away from the embryo. This process was first restricted to a collar of cells immediately surrounding the blastopore; later it spread to the ectoderm in general, after which the embryo died (Fig. 4). This set of events—cessation of development in circular blastopore stage, followed by degeneration of blastopore lips and death of the embryo—occurred uniformly in all embryos exposed to 10.0 mgm. per 100 cc. DBAS.

Embryos in concentrations of 2.13 and 4.26 mgm. per 100 cc. gastrulated completely but at a definitely reduced rate (Fig. 3). Neural folds developed, but in the embryos exposed to the stronger concentration (4.26) they degenerated before they could fuse to form a tube. This degeneration involved the same pigment recession and swelling of cells observed earlier in the cells of the blastoporal lips of embryos exposed to 10.0 mgm. per 100 cc. solutions of DBAS. In the case of the neural folds, the crest cells were affected first. The degeneration then spread to the remainder of the folds. The ectoderm remained alive until the neural fold tissue was completely degenerated and consisted of nothing but a large mass of pale swollen cells (Fig. 5). Following this complete neural degeneration, the ectoderm was also affected and the embryo died.

Fusion of the neural folds in embryos exposed to 2.13 mgm. per 100 cc. solutions of the carcinogen was retarded by about 8 hours as compared with the normal control embryos. In a few cases the folds failed to fuse in the hind brain region, and degeneration occurred in the exposed part. However the majority of the embryos in this concentration (2.13) completed neurulation. Thereafter they developed satisfactorily, but at a reduced rate. Growth rate, in terms of increase in length, was significantly retarded (Fig. 6). Morphological development also was delayed (Fig. 7) but appeared not to be significantly altered in relation to growth rate. Development of function, however, seemed to be more retarded than either growth rate or rate of morphological development. Thus, while normal embryos at stage 18 (4 mm.) undergo muscular contraction in response to mechanical stimulation, the embryos exposed to 2.13 mgm. per 100 cc. DBAS gave no muscular response at the same stage. In these latter animals, muscular activity could not be elicited until they were at stage 18.5 to 19 (4.5 to 5

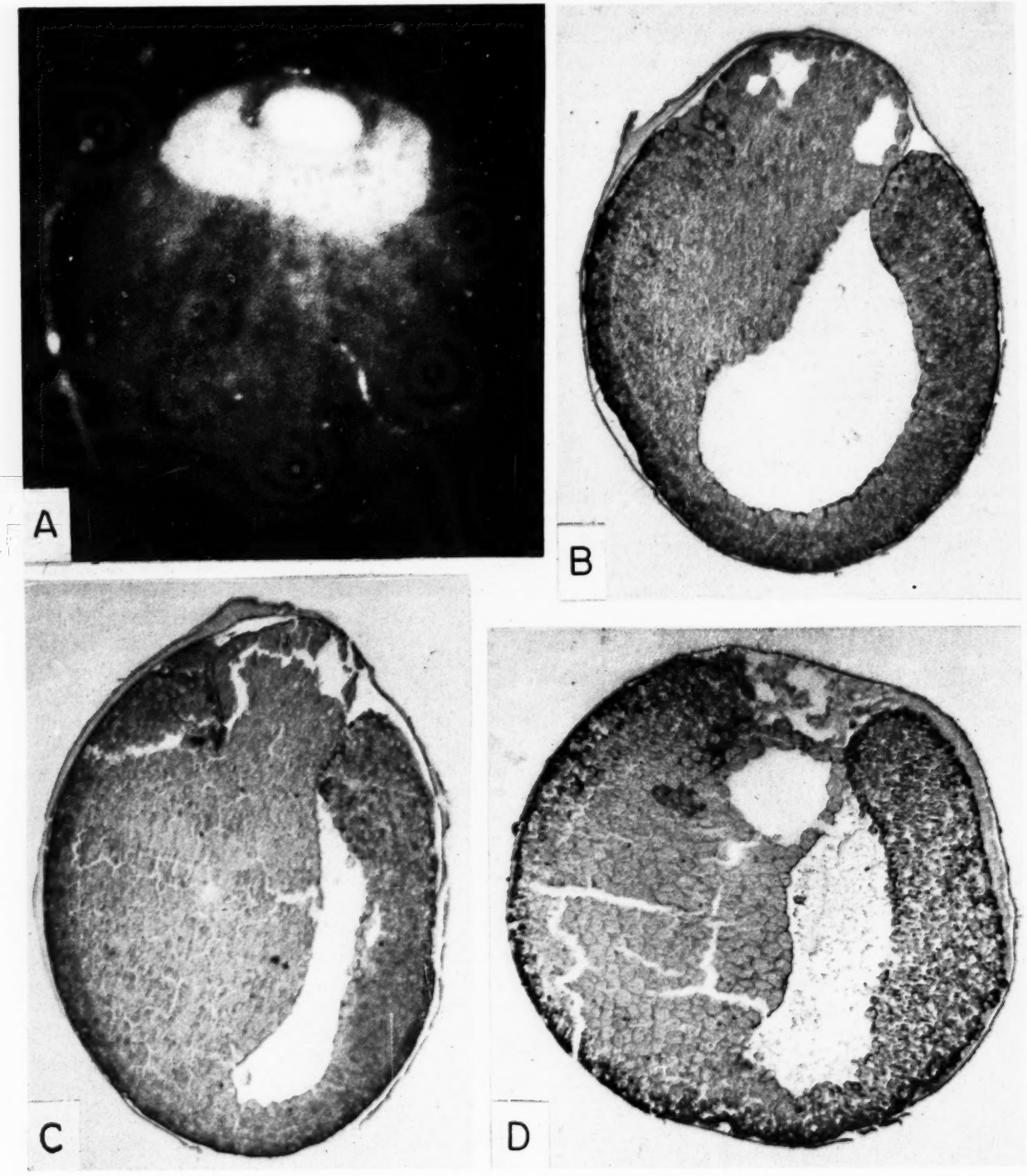

FIG. 4.—Degeneration in the frog's egg as a result of continuous exposure to a 10.0 mgm. per 100 cc. solution of DBAS. Mag. about × 35.

A. Living egg, showing moderate degeneration. A "collar" of pale, swollen, degenerated cells may be seen surrounding the persistent yolk plug.

B, C, and D. Sections showing progressive stages of degeneration. B, earliest stage. A single swollen cell may be seen at the ventral lip of the blastopore. The dorsal lip is normal. C, moderate degeneration. The demarkation of a "collar" of degenerated cells may be seen at one side. D, complete degeneration. Pycnotic nuclei and rounded up superficial cells may be seen. The yolk is relatively unaffected.

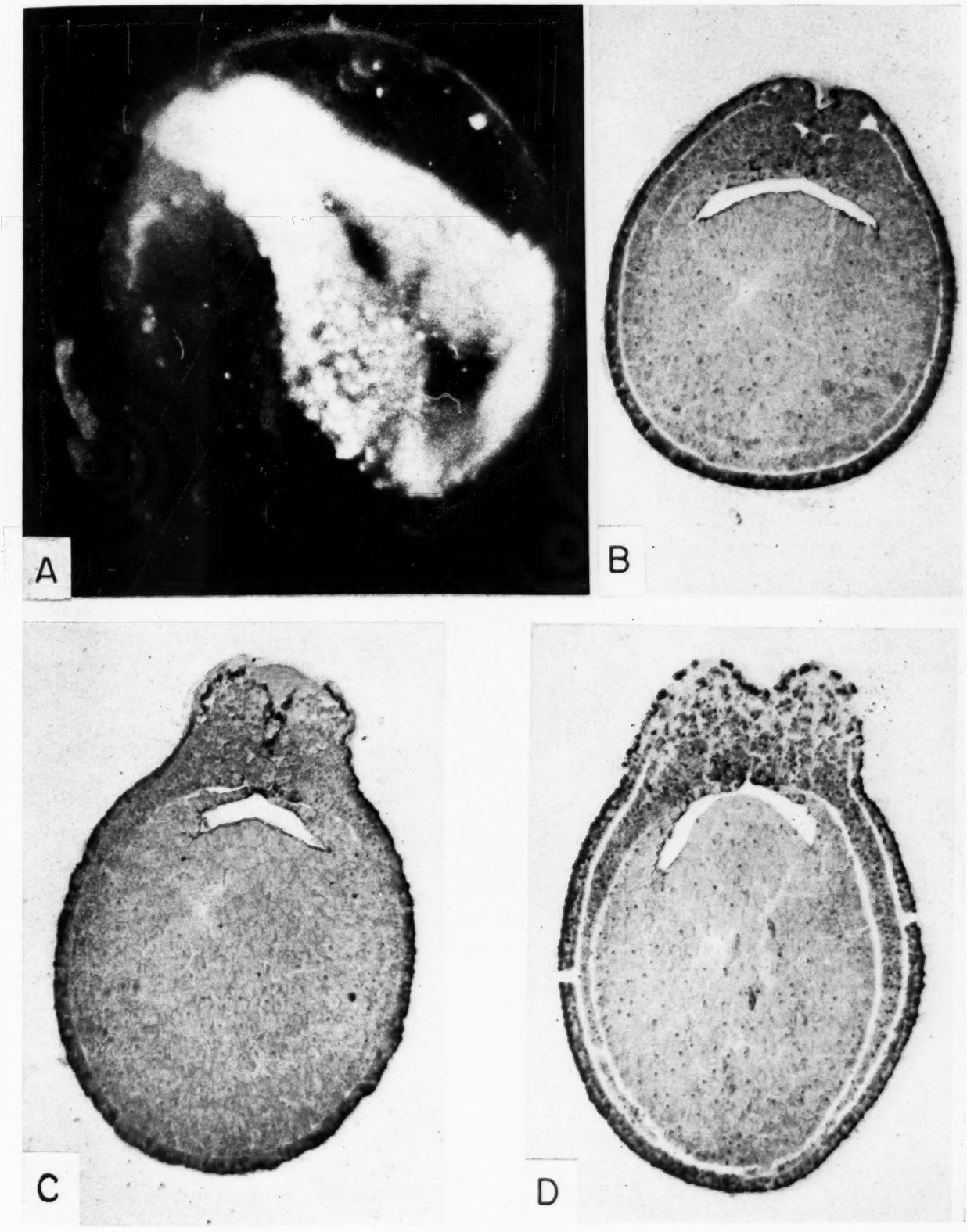

FIG. 5.—Degeneration in the frog's egg, as a result of continuous exposure to a 4.26 mgm. per 100 cc. solution of DBAS. Mag. about × 35.

A. Living egg showing degeneration of neural fold tissue. Characteristic swelling of cells and pigment recession may be seen.

B, C, and D. Sections showing progressive stages of degeneration. B, an isolated swollen cell, at the neural crest region, may be seen protruding beyond the normal margin of the neural fold. C, degeneration has affected the surface of the neural folds. Some pigment recession and cytolysis may be seen. D, degeneration has spread more deeply to include notochord and adjacent mesoderm. Rounding up and breaking away of cells may be seen. It is only after neural fold destruction is complete that body ectoderm becomes affected.

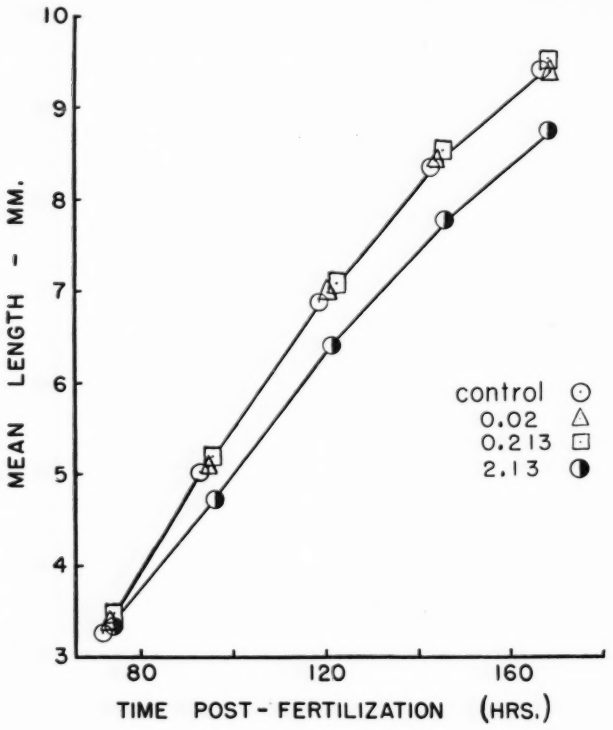

FIG. 6.—Growth curves of animals exposed continuously to solutions of DBAS, exposure beginning one hour after fertilization. Each point on the graph represents the mean length of 20 animals. Variation in length is ± 0.25 mm. The reduction of growth rate of embryos exposed to the 2.13 mgm. per 100 cc. solution is therefore significant.

mm.). Also, at later stages, the embryos exposed to carcinogen succumbed about twice as quickly as the controls when anesthetized for the purpose of making growth measurements. These observations on behavior suggest that the development of function in the nervous system or muscle tissue or both may be differentially retarded by the carcinogen.

II. The Relative Susceptibility to DBAS during Different Stages of Development

The results described above show that when eggs are placed in the carcinogen one hour after fertilization there are no effects on development until after the initiation of gastrulation, which occurs (at 19.6° C.) about 21 hours after fertilization. This may mean that (a) a latent period of 20 hours is required before any effects are forthcoming, or (b) the early embryo is immune to the carcinogen while the gastrula and subsequent stages are susceptible. Our results also show a retardative effect on post-neurula growth and development, which can be interpreted as either (a) simply the result of deleterious effects on gastrulation and neurulation or (b) a direct effect on post-neurula developmental processes themselves. The following experiments were designed to determine which of these interpretations, in each case, was correct.

Exposure of ovarian eggs to the carcinogen (380 eggs).—The 2 females which were to provide eggs

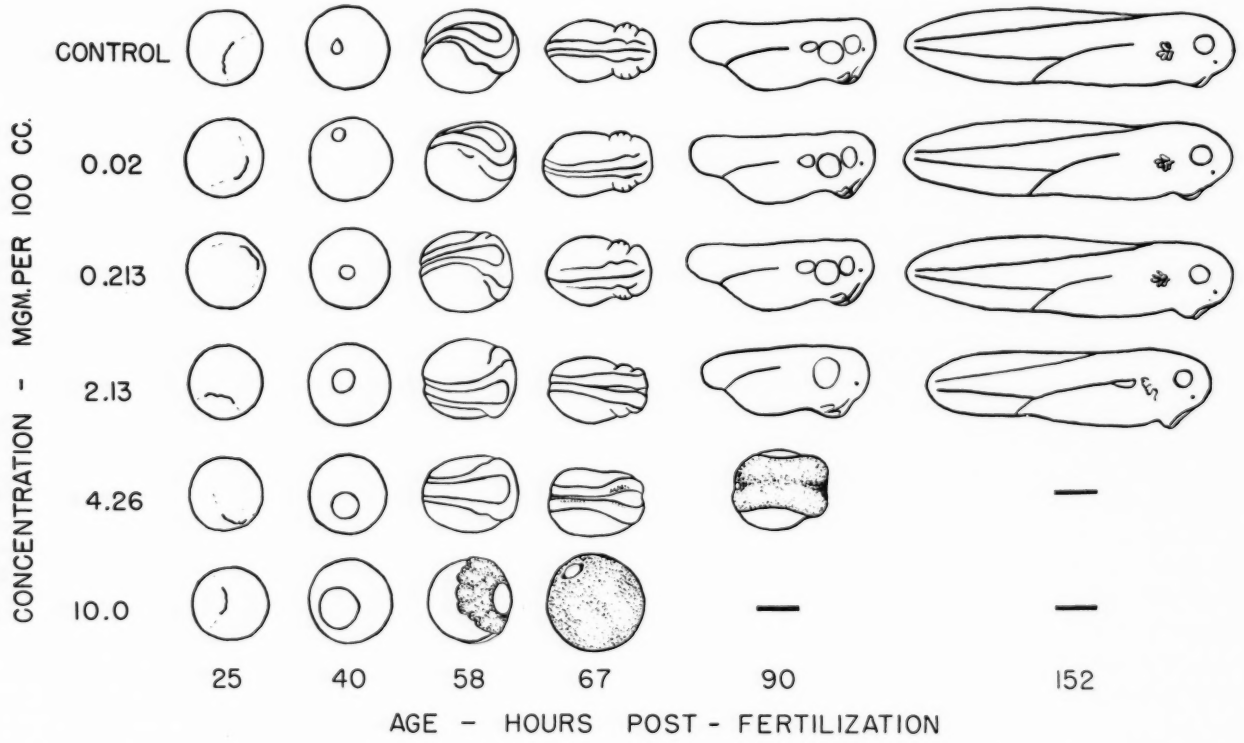

FIG. 7.—Summary of the effects of a water-soluble derivative of 1,2,5,6-dibenzanthracene on the early development of the frog. Mag. × 4.7.

for this experiment were each injected intraperitoneally with 1 mgm. of DBAS (in 1 cc. Ringer's solution). Fertilized eggs were obtained from the animals by pituitary injection 4 and 8 days later, respectively. Cleavage and later development were observed carefully to determine if the pre-fertilization exposure to the carcinogen had exerted any effect on the eggs. Development was normal throughout, indicating that ovarian eggs and those undergoing ovulation and maturation are not acted upon by the carcinogen, at least under the conditions of our experiment.

Exposure to DBAS during fertilization (72 eggs).—A sperm suspension was made up in 10 per cent Ringer's solution, containing 10.0 mgm. per 100 cc. of DBAS. The eggs were fertilized in the carcinogen and left in it for 2 hours. They were then transferred to 10 per cent Ringer's solution. All the eggs so treated developed perfectly normally throughout, indicating that the carcinogen, as used in this experiment, has no deleterious effect on the sperm or on the process of fertilization.

Exposure during cleavage and blastula formation, to the beginning of gastrulation (105 eggs).—The eggs in this experiment were exposed to a 10.0 mgm. per 100 cc. solution of the carcinogen from one hour after fertilization through the initiation of gastrulation 25 hours later. At this time the experimental and control embryos had both developed small crescentic blastopores. The experimental embryos were then removed from the carcinogen and placed in 10 per cent Ringer's solution. Subsequent development, through completion of gastrulation, neurulation, and post-neurula stages (through stage 25) was normal in both experimental and control embryos. This result shows that the pre-gastrula embryo not only undergoes no visible changes while it is exposed to the carcinogen, but that there are no invisible effects which become evident during later development.

Exposure during gastrulation (50 eggs).—In this experiment, the converse of the previous one, all eggs were allowed to develop normally in 10 per cent Ringer's solution through the beginning of gastrulation. The experimental embryos were then transferred to 10.0 mgm. per 100 cc. DBAS. Subsequently they were notably retarded and underwent typical degeneration of the lips of the blastopore, followed by death of the embryo. The effects were therefore the same as those observed in embryos exposed continuously (beginning one hour post-fertilization) to the carcinogen. This result, together with that described in the preceding paragraph, shows that there is a development of sensitivity to the carcinogen during gastrulation. This must take place specifically as a result of the changes in the animal during the gastrulation process, because previous exposure or lack of exposure to the carcinogen has no influence on the result.

Exposure during post-neurula development (40 eggs).—The embryos in this experiment were allowed to develop normally in 10 per cent Ringer's solution through neurulation. When the neural folds were completely fused (stage 16) three sets of embryos were placed in 4.26, 2.13, and 0.213 mgm. per 100 cc. solutions of DBAS respectively. The embryos in the strongest solution (4.26) were severely

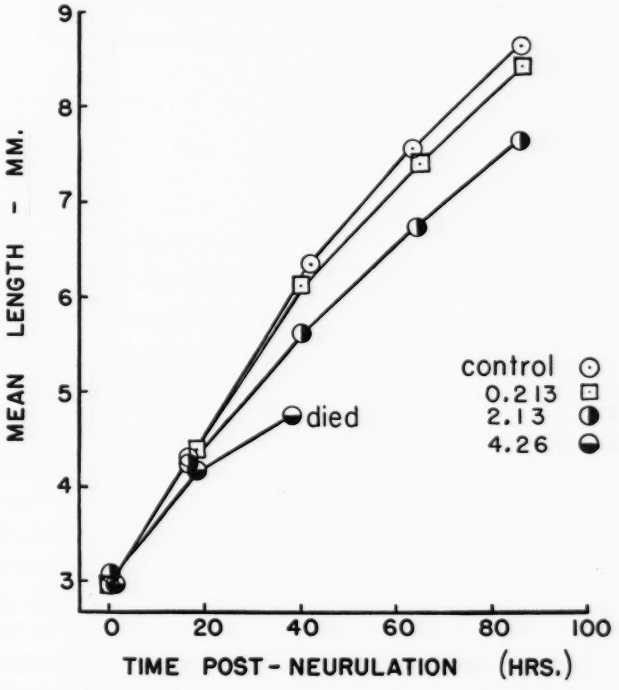

FIG. 8.—Growth curves of animals exposed to DBAS solutions during post-neurula development. Each point on the graph represents the mean length of 10 animals. Variation in length is ± 0.1 mm. to 0.2 mm. (except for length values of 2.13 animals at 63 and 85 hours, where it is approximately ± 0.4 mm.). The reduction in growth rate of embryos exposed to 4.26 and 2.13 mgm. per 100 cc. solutions of DBAS is unquestionably significant. The apparent slight reduction in rate of growth of embryos in 0.213 mgm. per 100 cc. DBAS is not significant. Other experiments have also shown that 0.02 mgm. per 100 cc. DBAS has no effect on growth rate.

retarded in growth rate during the first day of exposure (Fig. 8). During the 2nd day the actively outgrowing tails of these animals underwent a degeneration comparable with that occurring in the blastoporal tissue and neural folds of embryos exposed to the carcinogen during earlier stages of development. At 40 hours after subjection, the tail degeneration had extended cephalad onto the body causing the death of all embryos.

Embryos exposed to the 2.13 mgm. per 100 cc. solution of DBAS were definitely retarded in growth rate, the degree of retardation being of the same order as that occurring in animals continuously exposed to

the same concentration of the carcinogen from the beginning of development (compare Figs. 6 and 8). We observed also the same differential retardation of the development of reflexes as was described in section I of this report.

The weakest solution (0.213 mgm. per 100 cc. DBAS) had no significant effect on post-neurula development.

This experiment demonstrates that the post-neurula embryo is definitely sensitive to the carcinogen, and that the effects observed are not dependent on deleterious effects produced during gastrulation and neurulation.

Table I: Surface Tension Values of Some of the Solutions Used in These Experiments

Each value is an average of 10 or more readings by means of a du Noüy tensimeter. Variation in the case of 10.0 mgm. per 100 cc. DBAS was ± 3.1 dynes per cm. In the case of all other solutions it did not exceed ± 0.6 dynes per cm. Columns 2 and 3 show that surface tension-lowering capacity of a solution is not correlated with capacity to bring about detrimental effects upon development.

Solution	Surface tension, dynes per cm.	Effect on development of the embryo
H_2O	73.3	None
10 per cent Ringer's solution	73.2	None
Na-succinate 7.02 mgm. per 100 cc.*	72.5	None
Anthracene-succinate 7.46 mgm. per 100 cc.*	72.0	None
0.02 mgm. per 100 cc. DBAS	72.1	None
0.213 mgm. per 100 cc. DBAS	72.8	None
2.13 mgm. per 100 cc. DBAS	66.9	Slight retardation of growth
4.26 mgm. per 100 cc. DBAS	64.1	Moderate retardation. Degeneration beginning at neural fold stage
10.0 mgm. per 100 cc. DBAS	59.5	Extreme retardation. Degeneration beginning at yolk plug stage
Aerosol O.T. 0.2 mgm. per 100 cc.	59.0	None
Anthracene-aerosol 7.46 (anthracene) and 0.2 (aerosol) mgm. per 100 cc.	59.8	None

* Equimolar with 10.0 mgm. per 100 cc. concentration of DBAS.

III. The Specificity of the Effects of DBAS on Frog Development

The experiments to be described in this section were made to determine whether or not the effects of DBAS on frog development are due specifically to its carcinogenic properties. In each of these experiments the effects produced by a given substance (chosen for reasons given below) have been compared with those produced by a 10.0 mgm. per 100 cc. solution of DBAS. Exposure to the test substances was continuous, beginning one hour after fertilization. The development of the eggs was followed to stage 25 (11 mm.).

Na-succinate (100 eggs).—Embryos exposed continuously to a 7.02 mgm. per 100 cc. solution of Na-succinate (equimolar with 10.0 mgm. per 100 cc. DBAS) developed perfectly normally, while those exposed to 10.0 mgm. per 100 cc. DBAS underwent the usual retardation and degeneration during gastrulation. Therefore the succinate part of the DBAS molecule is not responsible for the effects produced by this carcinogen.

Na-anthracene-9,10-endo-α,β-succinate (cis), (90 eggs).—Anthracene was the only readily available related noncarcinogen of which the endo-succinate could be formed. The *cis* form of this substance is readily soluble (37). Embryos exposed continuously to a 7.46 mgm. per 100 cc. solution of anthracene-succinate (equimolar with 10.0 mgm. per 100 cc. DBAS) developed normally throughout, while the usual effects were produced by the DBAS. This fact, together with the results from the Na-succinate experiment, supports the hypothesis that the carcinogenicity of the DBAS is responsible for the effects observed.

Aerosol O.T. (100 eggs).—It was noticed in making up the DBAS solutions that the higher concentrations showed considerable frothing, which was taken as evidence of reduced surface tension. Subsequent measurements with a du Noüy tensimeter showed that the effective DBAS solutions have surface tensions significantly lower than that of 10 per cent Ringer's solution, while solutions of Na-succinate, anthracene-succinate, and dilute solutions of DBAS, none of which affect development, have surface tension values approximately the same as that of 10 per cent Ringer's solution, or water (Table I).

These measurements reveal a parallelism between the activity of the DBAS solutions and the degree of reduction of surface tension. The surface tension-reducing power of carcinogens has been noted by other workers and a connection between surface ten-

sion reduction and carcinogenesis has been postulated (4, 34). These considerations led us to expose eggs to aerosol O. T. (dioctyl ester of Na-sulfosuccinate), a powerful surface tension-reducing agent which has been found to be relatively nontoxic (22). A solution of aerosol with approximately the same surface tension as that of 10.0 mgm. per 100 cc. DBAS had no effects on development, indicating that the surface tension-reducing power of the carcinogen is not alone responsible for its activity.

Anthracene-aerosol (105 eggs).—Although anthracene alone had no harmful effect, and lowered surface tension alone had no effect, the experiment described above did not exclude the possibility that surface tension reduction is a contributory factor which, in combination with the special molecular configuration of a carcinogen, accounts for its activity. We attempted an experimental evaluation of this latter hypothesis by diluting a solution of the non-carcinogen, anthracene-succinate (which has a molecular configuration related to that of DBAS but which does not reduce surface tension), with aerosol. The solution so made had a surface tension value and a molar concentration of anthracene-succinate equal to those of 10.0 mgm. per 100 cc. DBAS. Eggs exposed to this solution developed perfectly normally, while those exposed to 10.0 mgm. per 100 cc. DBAS died as usual during the latter part of gastrulation.

This experiment may be taken as additional indication that the activity of DBAS is due to its carcinogenicity.

Metabolic poisons (120 eggs).—Carcinogens have been found in certain cases to inhibit respiration and glycolysis (7, 26, 27) and thus appear to have some properties in common with respiratory poisons. This consideration suggested that respiratory poisons might affect frog embryos in the same way as the carcinogen. We examined this possibility by exposing developing eggs continuously to (a) M/10,000 KCN, (b) M/1,000 phenylurethane, and (c) M/1,000 iodoacetate. In these concentrations KCN and iodoacetate allowed development to proceed normally from fertilization through stage 25. The phenylurethane caused retardation and gross morphological abnormalities, but produced no specific cellular degeneration such as was a constant feature in the experiments with DBAS. Other workers (5, 6, 8) have found that KCN and phenylurethane, in concentrations ranging from M/1,000 to M/10,000, bring about retardation and the development of abnormalities in the frog's egg. To our knowledge, however, no one has recorded amongst the effects of these substances the specific set of effects produced by DBAS in our experiments.

Salts and miscellaneous substances (120 eggs).—Jenkinson (19) subjected frog eggs and embryos to 36 different substances. Some of these (0.625 per cent NaCl, 1.09 per cent NaBr, 0.77 per cent $MgCl_2$, and 6.6 per cent cane sugar) produced effects resembling those obtained by us with DBAS. These particular solutions are all hypertonic to the frog egg and early embryo (2) and the effects observed were possibly due to this hypertonicity rather than to any specific properties of the chemicals. However, in order to check on the apparent similarity of effects, we repeated Jenkinson's experiments by exposing developing eggs continuously to the four substances in the same concentrations as are noted above.

In NaCl and NaBr the eggs became shrunken and distorted, and development ceased in mid-cleavage or late cleavage. In the sugar solution there was some attempt at gastrulation, but the eggs died without the formation of a well defined blastopore. The $MgCl_2$ was the only solution in which our results were in any way comparable with those of Jenkinson, in that the eggs survived through neurulation and some neural groove degeneration did occur. However, it occurred in only 6 of 20 animals and all these showed accompanying abnormalities. Thus although $MgCl_2$ does produce degeneration of neural tissue, the effect differs from that produced by DBAS in that the degeneration does not occur uniformly in all animals, and it is accompanied by a variety of structural abnormalities never found in embryos exposed to the carcinogen.

Effects of another carcinogen: Na-3-methylcholanthrene-6,12b-endo-α,β-succinate (trans), (120 eggs).—The experiments so far reported indicate that the effects produced by DBAS are specifically related to that compound, and furthermore that they might be specifically caused by its carcinogenic properties. In order to get confirmatory evidence on this latter point it was necessary to subject eggs to another water-soluble carcinogen and to observe (a) whether the effects would be the same in kind, and (b) whether there would be any correlation between potency of the carcinogen and severity of effect.

The parent hydrocarbon, 3-methylcholanthrene, was obtained from the Eastman Kodak Company. The succinic acid derivative was made by Mr. D. Robinson, of the Department of Chemistry, McGill University, according to the methods of Bachmann and Kloetzel (1) and Warren (37) and had a melting point of 190° C. (uncorrected). The Na salt was prepared as for DBAS.

The concentration used was 9.75 mgm. per 100 cc. (equimolar with 10.0 mgm. per 100 cc. DBAS), but owing to impurities (as evidenced by charred residue in the test tube) the effective concentration was reduced and the carcinogen acted not like a 10.0 but rather like a 4.26 mgm. per 100 cc. solution of DBAS.

However the important point is that, in the uniformity and specificity of its action, and in every detail, the effect caused by the methylcholanthrene-succinate was like that caused by DBAS.

DISCUSSION

The main points concerning the effects of DBAS on frog development may be summarized as follows: (a) Lack of stimulating effect on developmental rate in any concentration (0.02 to 10.0 mgm. per 100 cc.). (b) Retardation of developmental rate (after beginning of gastrulation) in stronger concentrations. (c) Specific destruction of the lips of the blastopore and, in weaker concentrations, of the neural folds and outgrowing tail bud. (d) Differential retardation of the capacity for muscular movement during post-neurula development. (e) Lack of effect on the morphological organization of the embryo.

This set of effects is produced by another water-soluble carcinogen, methylcholanthrene-succinate, but by none of the noncarcinogenic substances tried by us. Also, while there are records in the literature of substances or treatments which bring about one or another of these effects, we know of no noncarcinogenic substance which will produce the combination of effects listed above. This suggests that these effects may be produced specifically by substances with carcinogenic activity.

In regard to the question of the effect of the carcinogen on rate of growth, our results are in contrast to those of Creech (9, 10), who observed acceleration of growth rate in tissue cultures exposed to a concentration of 0.01 mgm. per cc. DBA-choleic acid (equivalent, in terms of the carcinogen, to our 0.213 mgm. per 100 cc. concentration of DBAS). They are also in contrast to the reports of Mottram (24), Wolman (39), Spencer and Melroy (33), Goldstein (15), Hopper and Clapp (18), Reimann and Hammett (28), Owen and his colleagues (25), and others, who have observed stimulative effects upon the exposure of various invertebrates to carcinogens.

Our results, however, conform with those of Haddow and his associates (16, 17, and previous papers), Badger and his group (3), and others who have observed a retardative effect of carcinogens on the growth of rats and of spontaneous and induced tumors. They also have points in common with those obtained by Reiss (29), who described degenerative changes in sea urchin eggs exposed to an emulsion of tar in sea water; and with those of Mauer (23) and Earle and Voegtlin (13, 14), who exposed tissue cultures to carcinogens and observed retardation and degeneration, but never acceleration.

Our observation that the carcinogen has no effect on morphological organization of the embryo is at variance with the work of Waddington (35, 36), Shen (31), and others, who reported that DBAS implanted in agar blocks acted as an "organizer" to induce the formation of axial structures in amphibian embryos. In a general criticism of "induction" by chemical compounds, Woerdeman (38) suggested that the induction with DBA reported by Waddington and others was only a secondary effect, the primary effect being cell injury which resulted in release of the embryo's own "organizer." Shen, in a paper following that of Woerdeman's, asserted that he found no evidence of injury in his experiments, and that the carcinogen must therefore be acting directly as an organizer. However, his most effective concentration of DBAS in the agar implants was approximately 5.0 mgm. per 100 cc. Thus the cells immediately adjacent to the implant presumably were exposed to this concentration, which, as we have shown above, causes degeneration of neural tissue.[2] Differences in results may be due to differences in the species of amphibian used, or to differences in the method of administering the carcinogen. None the less, it would appear that "induction" by means of carcinogens will have to be examined thoroughly before it can be accepted as one of the actions of these substances.

SUMMARY AND CONCLUSIONS

Eggs of the frog, *Rana pipiens,* were reared in solutions of Na-1,2,5,6-dibenzanthracene-9,10-endo-α,β-succinate (*trans*), the exposure beginning one hour after fertilization. The concentrations used were 0.02, 0.213, 2.13, 4.26, and 10.0 mgm. per 100 cc.

The results were as follows:

1. No evidence of stimulative effect in any concentration.

2. Retardation of developmental rate (after beginning of gastrulation) in the three stronger concentrations, the degree of retardation being proportional to the concentration.

3. Degeneration of embryos in the stronger concentrations, degeneration following always a definite uniform pattern and beginning always at the same developmental stage for a given concentration.

4. Differential retardation of capacity for muscular movement during post-neurula development.

5. Lack of any effect on the morphological organization of the embryo.

Various experiments (with a surface tension-reducing agent, Na-succinate, a related noncarcinogen, me-

[2] Shen, in his most recent paper (Neural Induction in Epidermal Explants in Liquid Medium. J. Exper. Biol., **19**:5-10. 1942), states that neural "induction" was obtained in isolated presumptive epidermis from *Amblystoma mexicanum* cultured 10 days in DBAS. It is not stated whether the *cis* or *trans* form of the carcinogen was used.

tabolic poisons, and miscellaneous substances) indicated that the effects noted above were specific for DBAS, and suggested further that the carcinogenicity of DBAS might be responsible for the effects. Treatment of the eggs with another water-soluble carcinogen, Na-3-methylcholanthrene-6,12b-endo-α,β-succinate, gave confirmatory evidence on this latter point.

REFERENCES

1. Bachmann, W. E., and Kloetzel, M. C. The Reaction between Maleic Anhydride and Polycyclic Hydrocarbons. J. Am. Chem. Soc., **60**:481-485. 1938.
2. Backman, E. L., and Runnström, J. Der osmotische Druck während der Embryonalentwicklung von Rana temporaria. Pflüger's Arch. f. d. ges. Physiol., **144**:287-345. 1912.
3. Badger, G. M., Elson, L. A., Haddow, A., Hewett, C. L., and Robinson, A. M. The Inhibition of Growth by Chemical Compounds. Proc. Roy. Soc., London, s.B, **130**:255-299. 1942.
4. Bauer, E. Theoretische und experimentelle Untersuchungen über die Entstehungsbedingungen des Carcinoms. Zugleich ein Beitrag zur Frage des konstitutionellen Momentes. Ztschr. f. Krebsforsch., **20**:358-374. 1923.
5. Bellamy, A. W. Differential Susceptibility as a Basis for Modification and Control of Early Development in the Frog. I. Biol. Bull., **37**:312-361. 1919.
6. Bellamy, A. W. Differential Susceptibility as a Basis for Modification and Control of Development in the Frog. II. Types of Modification Seen in Later Developmental Stages. Am. J. Anat., **30**:473-502. 1922.
7. Boyland, E., and Boyland, M. E. Studies in Tissue Metabolism. III. The Effect of Oxidised 1:2:5:6-Dibenzanthracene. Biochem. J., **28**:244-256. 1934.
8. Brachet, J. Etude du métabolisme de l'oeuf de Grenouille (R. fusca) au cour du développement. 1—La respiration et la glycolyse, de la segmentation à l'éclosion. Arch. de biol., **45**:611-727. 1934.
9. Creech, E. M. H. Carcinogenic and Related Non-Carcinogenic Hydrocarbons in Tissue Culture. I. Am. J. Cancer, **35**:191-202. 1939.
10. Creech, E. M. H. Carcinogenic and Related Non-Carcinogenic Hydrocarbons in Tissue Culture. II. Am. J. Cancer, **39**:149-160. 1940.
11. Dobrovolskaia-Zavadskaia, N. Les doses minimes de 1:2:5:6-dibenzanthracène capables de produire, en une seule injection sous-cutanée, un cancer chez la souris. Compt. rend. Soc. de biol., **129**:1055-1057. 1938.
12. DuShane, G. P., and Hutchinson, C. The Effect of Temperature on the Development of Form and Behavior in Amphibian Embryos. J. Exper. Zool., **87**:245-258. 1941.
13. Earle, W. R., and Voegtlin, C. The Mode of Action of Methylcholanthrene on Cultures of Normal Tissues. Am. J. Cancer, **34**:373-390. 1938.
14. Earle, W. R., and Voegtlin, C. A Further Study of the Mode of Action of Methylcholanthrene on Normal Tissue Cultures. Pub. Health Rep., **55**:303-322. 1940.
15. Goldstein, S. A Microbiological Test for Carcinogenic Hydrocarbons. Science, **86**:176-177. 1937.
16. Haddow, A., Scott, C. M., and Scott, J. D. Influence of Certain Carcinogenic and Other Hydrocarbons on Body Growth in Rat. Proc. Roy. Soc., London, s.B, **122**:477-507. 1937.
17. Haddow, A., and Robinson, A. M. Association of Carcinogenicity and Growth-Inhibitory Power in the Polycyclic Hydrocarbons and Other Substances. Proc. Roy. Soc., London, s.B, **127**:277-287. 1939.
18. Hopper, S. H., and Clapp, D. B. Effect of Carcinogenic and Other Hydrocarbons on Growth of *Escherichia communior*. J. Bact., **38**:13-23. 1939.
19. Jenkinson, J. W. On the Effects of Certain Solutions upon the Development of the Frog's Egg. Arch. f. Entwcklngsmechn. d. Organ., **21**:367-460. 1906.
20. Lettinga, T. W. De carcinogene werking van kleine doses 1:2:5:6-dibenzanthraceen. Dissertation, Amsterdam. 1937. (Table of results given in Fieser, L. Am. J. Cancer, **34**:49. 1938.)
21. Levine, M., and Bergmann, H. The Cytology of the 1,2,5,6-Dibenzanthracene Mouse Tumors. Am. J. Cancer, **39**:504-520. 1940.
22. Lorenz, E., Shimkin, M. B., and Stewart, H. L. Preparations of Dispersions of Carcinogenic Hydrocarbons and Hormones with the Aid of Dioctyl Ester of Sodium Sulfosuccinate (Aerosol O. T.). J. Nat. Cancer Inst., **1**:355-360. 1940.
23. Mauer, G. Untersuchungen über die Einwirkung kanzerogener Kohlenwasserstoffe auf Gewebekulturen. Arch. f. exper. Zellforsch., **21**:191-211. 1938.
24. Mottram, J. C. An Increase in the Rate of Growth of Paramecium Subjected to the Blastogenic Hydrocarbon, 3:4-Benzpyrene. Nature, London, **144**:154. 1939.
25. Owen, S. E., Weiss, H. A., and Prince, L. H. Carcinogenics and Growth Stimulation. Science, **87**:261-262. 1938.
26. Pourbaix, Y. Etude de l'influence d'un corps cancérigène, le 1-2 5-6 dibenzène anthracène, sur la respiration de la cellule normale. Compt. rend. Soc. de biol., **112**:1222-1225. 1933.
27. Pourbaix, Y. Respiration des coupes de cerveau *in vitro* en présence d'un corps pur cancérigène, le 1-2 5-6 dibenzène anthracène. Compt. rend Soc. de biol., **113**: 930-933. 1933.
28. Reimann, S. P., and Hammett, F. S. The Proliferation Stimulating Action of 1:2:5:6-Dibenzanthracene on *Obelia geniculata*. Am. J. Cancer, **23**:343-349. 1935.
29. Reiss, P. Sur des anomalies du développement de l'oeuf d'oursin par l'action du goudron et leurs rapports avec les phénomènes cytologiques de la pathogénie du cancer. Arch. de biol., **34**:345-368. 1924.
30. Rothlin, E. M.S. 222 (Lösliches Anaesthesin), ein Narcoticum für Kältblüter. Schweiz. med. Wchnschr., **62**: 1042. 1932.
31. Shen, S. C. A Quantitative Study of Amphibian Neural Tube Induction with a Water-Soluble Hydrocarbon. J. Exper. Biol., **16**:143-149. 1939.
32. Shumway, W. Stages in the Normal Development of *Rana pipiens*. I. External Form. Anat. Rec., **78**:139-147. 1940.
33. Spencer, R. R., and Melroy, M. B. Effects of Carcinogens on Small Free-Living Organisms. I. *Eberthella typhi*. J. Nat. Cancer Inst., **1**:129-134. 1940.
34. Traube, J. Bemerkungen zu der Arbeit von Katzenstein und Knake über die Anregung des Epithelwachstums bei gleichzeitiger Störung des Bindgewebswachstums durch oberflächenaktive bzw. grenzflächenaktive Stoffe in Gewebekulturen. Ztschr. f. Krebsforsch., **33**:411. 1931.
35. Waddington, C. H. Studies on the Nature of the Amphibian Organization Centre. VII. Evocation by Some Further Chemical Compounds. Proc. Roy. Soc., London, s.B, **125**:365-372. 1938.

36. WADDINGTON, C. H., and NEEDHAM, D. M. Studies on the Nature of the Amphibian Organization Centre. II. Induction by Synthetic Polycyclic Hydrocarbons. Proc. Roy. Soc., London, s.B, **117**:310-317. 1935.

37. WARREN, F. L. XIX. The Action of Some Endosuccinic Acids Derived from Polycyclic Hydrocarbons on the Red Blood Corpuscles of the Mouse. Biochem. J., **33**:165-169. 1939.

38. WOERDEMAN, M. W. Embryonic "Induction" by Chemical Substances. Koninklijke Akad. van Wetenschappen te Amsterdam. Proceedings of the Section of Sciences. **39**: 306-314. 1936.

39. WOLMAN, M. A Proliferative Effect of Carcinogenic Hydrocarbons upon the Multiplication of Paramecia. Growth, **3**:387-396. 1939.

The Inheritance of Susceptibility to Tumors Induced in Mice

I. Tumors Induced by Methylcholanthrene in Five Inbred Strains of Mice*

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In this study the susceptibility to tumors induced with a single subcutaneous injection of methylcholanthrene has been determined for five inbred strains of mice (5). The work was undertaken in order to accumulate control data and to investigate the inheritance of this susceptibility. The results obtained with the F_1 and backcross progeny of the C3H and JK strains will appear in subsequent reports.

Origin of the Strains

The CBA, C3H, CHI, NH, and JK were the strains used (16). The CHI, C3H, and CBA strains were derived as substrains from mice that were the progeny of a single cross between the A and D strains of unpedigreed mice. They were carried as single brother-to-sister matings or as a backcross to the immediate parent when siblings were not available. The JK strain originated as the F_1 of the cross of J and K unpedigreed parents. When the CBA strain had been inbred in the above manner for 31 generations a CBA female was crossed to a male of the N strain after it had been pedigreed by inbreeding for 20 generations. The resulting CBAN strain was inbred for 9 generations, and a CBAN female was crossed to a male of the JK strain that had then been inbred for 29 generations. The progeny comprised the NH strain (15). The origin and relationship of the strains is given in Fig. 1. The figures appearing below the strain letters denote the number of generations each strain had been inbred when used for this experiment.

METHODS

The mice were isolated, 6 or less in each side of a box, and were not allowed to breed. The number of mice used may be found in Table I. At 60 days of age, 1 mgm. of methylcholanthrene in 0.1 cc. of oil of benne (sesame oil) was injected subcutaneously into the right flank of each mouse. The oil had previously been tested and found to be not carcinogenic. The mice were given a diet of nurishmix and lettuce with unlimited water and kept in a room at 70° F. with a humidity of 50 to 60 per cent. After injection they were palpated for tumors at intervals not greater than 14 days. Cysts were broken if they formed. The palpation was done by the same observer throughout the experiment, and the time at which the tumors first appeared was recorded. This record was made permanent only if the tumor continued to grow, as determined subsequently. Some of the tumor-bearing mice were killed and sections of the tumors taken at right angles to the surface and continuing through the base. The tumors were fixed in Bouin's fluid and stained with hematoxylin and eosin. An autopsy was performed on each mouse killed. The survival times of the remaining mice were recorded. The experiment was continued until all the mice either had died or had developed tumors.

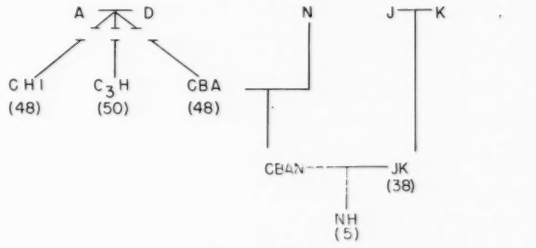

Fig. 1.—Origin of the strains.

RESULTS

The final percentage of mice developing tumors, the time from injection to tumor appearance (induction time), the time from tumor appearance to the death of the mice (survival time), and the types of neoplasia as judged by histological observation have been used as criteria for the differences among the

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strains. The mean and the median induction times appear in Table I. The median in these types of data is probably the more reliable measure since a few mice developing tumors late may cause the average value to be misleading. Since mice are dying from other causes throughout the period during which they are developing tumors, curves were plotted to show the total number of mice which had developed a tumor at a given time calculated as the percentage of the number alive at that time. The numbers appearing on the abscissa represent the number of days from the time of injection to the time of tumor appearance. The mice were observed in most instances for a period longer than one year, but data for that time only are given in the figures because any additional time does not alter the conclusions drawn.

The cumulative curves of tumor appearance assume the sigmoid form. In Figs. 2 to 6 the curves of tumor incidence in males compared to females are given for the five strains. Superimposition of these curves does not present any change in the relationship of the strains with respect to tumor induction time, although beyond 200 days the curves for NH males and JK females overlap. There is no sex difference in susceptibility to tumors induced with this dosage of methylcholanthrene in the C3H, CBA, NH, and JK strains. The induction time in CHI males and females does differ throughout the course of the curve (Fig. 4), and there is a difference of 23 days between the average induction times of the two. The difference in the medians is 8 days. This difference is suggestive and warrants further study. The use of a smaller dose of carcinogen would possibly bring out a sex difference more clearly as Sall and Shear (13) have found. The value for the median is lower than that for the average induction time in each case. There is closer agreement between the median values for the sexes as compared to the mean values for all the stocks except the JK strain (Table I). The survival times in ascending order are those of the JK, CBA, C3H, CHI, and NH strains. The interrelationship of the strains is not the same for tumor induction time as for survival time. The mean survival time is greater for females than for males.

TABLE I: INDUCTION AND SURVIVAL TIMES FOR MICE INJECTED WITH METHYLCHOLANTHRENE

Strain		Number of mice injected		Tumors		Died without tumor after first tumor appeared	Percentage dying without tumors		Induction time in days		Survival time with tumor		
									Mean	Median	No.	Mean days	
CBA	Males	66	184	60	173	6	9.1	6.0	98.8	95	16	36.0 ± 2.2	39.7
	Females	118		113		5	4.2		95.2	95	55	40.8 ± 1.6	
									96.6				
C3H	Males	83	177	79	167	3	3.7	4.0	72.2	64	15	35.9 ± 2.5	39.9
	Females	94		88		4	4.3		65.7	63	52	40.8 ± 1.2	
									68.6				
CHI	Males	133	253	105	204	28	21.2	18.4	86.6	84	27	40.5 ± 2.0	44.2
	Females	120		99		18	15.4		109.6	92	38	47.2 ± 2.0	
									99.2				
JK	Males	77	190	53	138	23	30.2	26.9	140.2	132	6	31.0 ± 0.8	34.8
	Females	113		85		28	24.8		149.4	143	30	35.4 ± 1.3	
									145.8				
NH	Males	137	254	97	194	34	25.9	21.8	136.4	115	22	43.8 ± 2.6	45.4
	Females	117		97		20	17.1		125.3	111	39	46.7 ± 2.1	
									133.1				
	Total	1,058		876		169					300		

Curves of the C3H, NH, and JK strains do not overlap (Fig. 7). The CHI and CBA strains present overlapping curves. Clearly the C3H and JK strains are the most and least susceptible respectively, with average induction times of 68.6 and 145.8 days and final tumor percentages of 96.0 and 73.1 per cent (Table I). There is no apparent difference in tumor induction time between the CBA and CHI strains, but there is a difference between these two strains and the other three. The NH strain is the fourth most susceptible strain; it has an average induction time of 133.1 days. The percentage values are based on a total of 1,058 mice.

Values for the total number of mice with tumors are in the same sequence as the curves presented in Fig. 7. However, the percentage of mice failing to

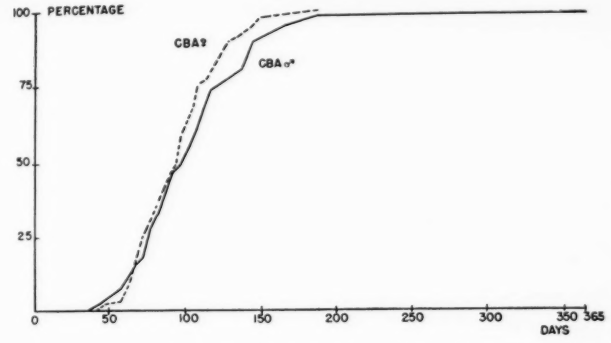


FIG. 2.—Time from injection to appearance of tumors in CBA mice.

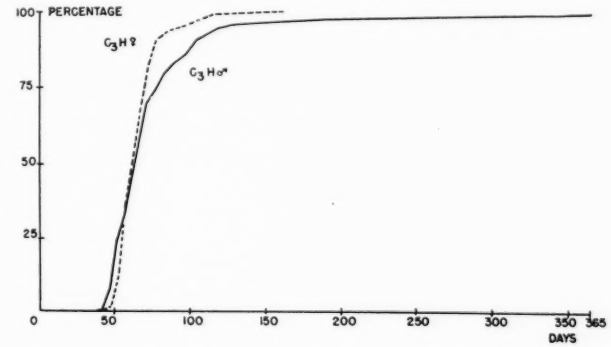


FIG. 3.—Time from injection to appearance of tumors in C3H mice.

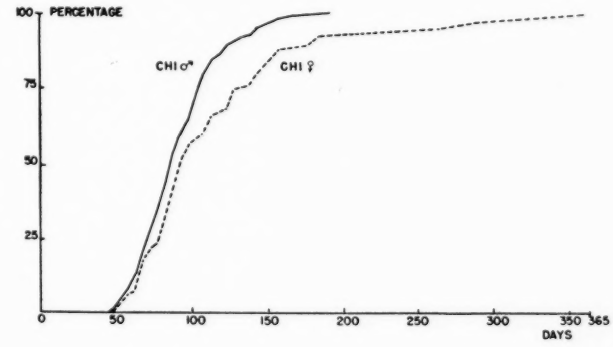


FIG. 4.—Time from injection to appearance of tumors in CHI mice.

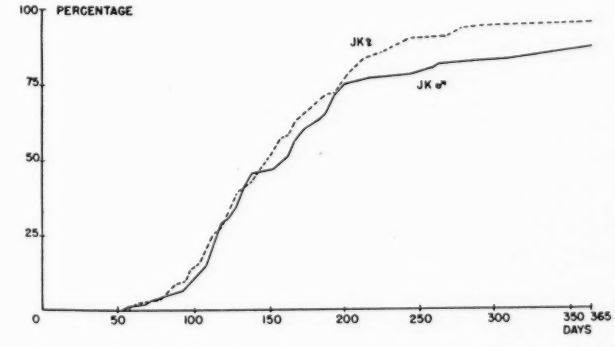


FIG. 5.—Time from injection to appearance of tumors in JK mice.

develop tumors was 18.4 for the CHI and 5.97 for the CBA mice, whereas the curves of induction time of the two are very similar.

Tumors appeared at the site of injection and grew rapidly in every instance. Ulceration occurred in relatively few animals. Palpation revealed no striking difference in the consistency of the various tumors in the different strains. All the tumors were quite firm. Rarely did epilation occur over a tumor. A section through the tumors usually showed central necrosis after the neoplasm had been present for 2 weeks or longer. In rare instances cystic areas filled with bloody fluid or pus were discovered on section. The

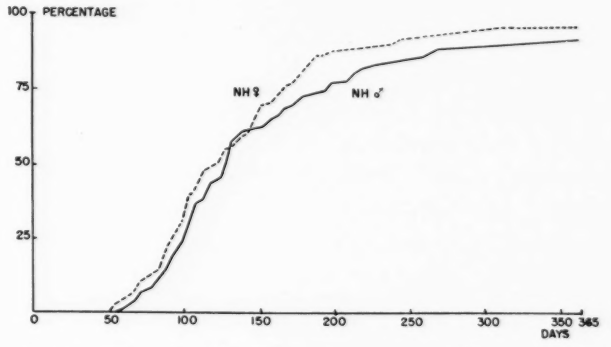


FIG. 6.—Time from injection to appearance of tumors in NH mice.

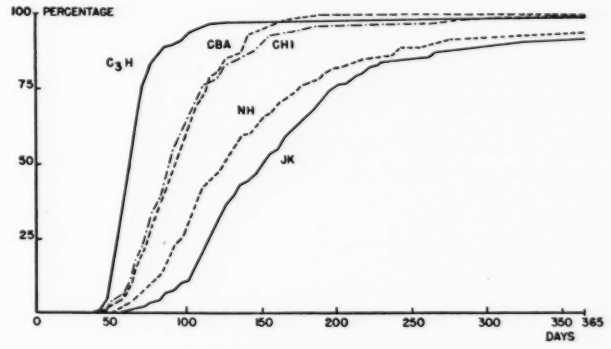


FIG. 7.—Time from injection to appearance of tumors in five inbred strains.

section for microscopic study was taken from only one area of the tumor.

Several different types of tumors were diagnosed in the group of animals treated with the carcinogen (Table II). Those occurring most frequently were spindle cell sarcomas and rhabdomyosarcomas. Epidermoid carcinomas were seen, and in one instance an anaplastic sarcoma was found. These four primary types contain all the kinds of cells seen throughout the series. In addition, various combinations of two or more of the four were seen. They are designated here as mixed tumors. The term simply means that in this type there were two or more of the primary types side by side in the same tumor. Those observed were

carcinoma-and-spindle cell sarcoma (Fig. 8), carcinoma-and-rhabdomyosarcoma, spindle cell sarcoma-and-rhabdomyosarcoma, spindle cell sarcoma-anaplastic sarcoma-and-rhabdomyosarcoma.

Invading malignant tissue or the carcinogen or both cause injury to the striated muscle. It was necessary to distinguish this injured muscle from the neoplastic process which results in a rhabdomyosarcoma (10). The rhabdomyosarcomas (Figs. 9 and 10) were bizarre in appearance. Several different types of cells were seen in them and occurred together or separately in a given tumor. Large syncytia, large and small round cells, foreign body giant cells, spindle cells, small polygonal cells, polymorphonuclear leukocytes, and blood sinuses were found in various combinations in these tumors.

The epidermoid carcinomas encountered showed typical pearl formation. There were abundant mitoses, particularly at the base. Identification was simple, and they were the easiest group to diagnose. Quite frequently in tumors other than carcinoma the overlying epidermis showed a pronounced hyperplasia with the stratum spinosum dipping deeply into the corium, and the granular layer was more prominent than usual. The sebaceous glands and hair follicles were likewise hyperplastic in some cases. The eosinophilia frequently found in the corium of mice painted with methylcholanthrene (6) was rare in the tumors of the injected animals. In only one case was it as pronounced as in the painted animals. Occasionally isolated eosinophils were seen in the corium and tumor tissue, but not in large numbers.

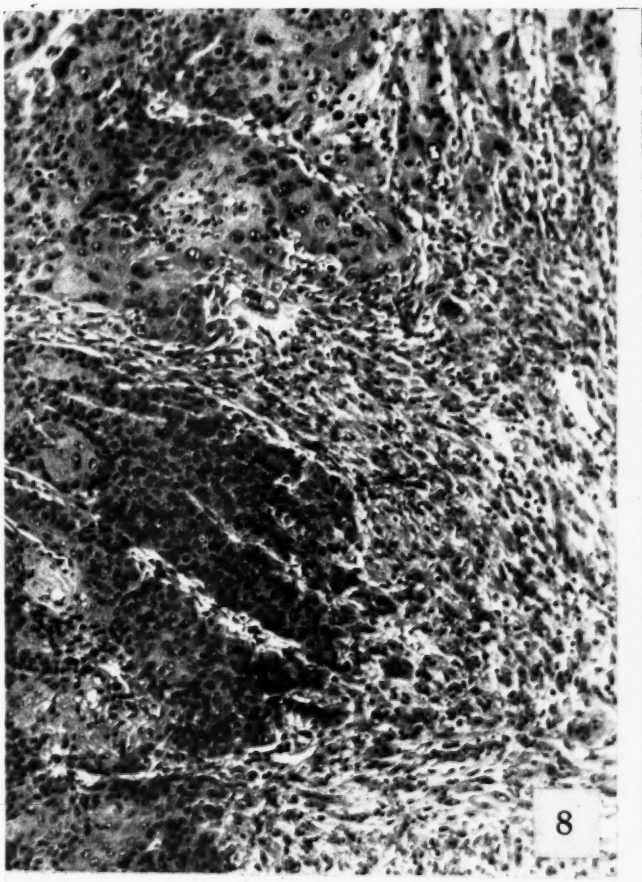
The spindle cell sarcomas (Fig. 11) include all those malignant tumors with spindle-shaped cells. In most instances these were arranged in criss-crossing bands which could sometimes be identified in the gross. Many mitotic figures were in evidence in these tumors. Within the group there was wide variation in morphology. No attempt was made to subdivide the group into fibrosarcomas, leiomyomas, and myoblastomas. One anaplastic sarcoma occurred in a C3H male. This tumor and the anaplastic portion of the mixed tumors consisted of small polygonal and round cells which stained heavily.

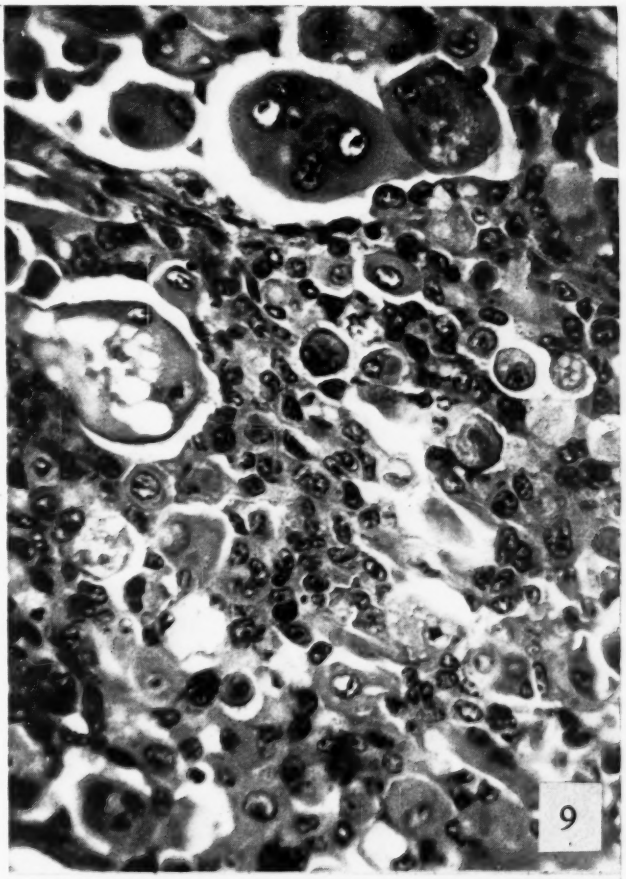
The spindle cell sarcomas and rhabdomyosarcomas were by far the most frequent neoplasms in all groups. The relative percentage of each was not constant. Rhabdomyosarcomas were more frequent in C3H and CHI mice, spindle cell sarcomas in CBA and JK mice. The types of tumors occurring in the NH strain and an account of the establishment of substrains has been published by Strong (15). Epidermoid carcinoma appeared only in the CBA mice. The anaplastic sarcoma in the C3H male has been mentioned. Of the mixed tumors, the spindle cell sarcoma-and-rhabdomyosarcoma was the most frequent type except in the C3H strain. The average induction time of spindle cell sarcoma was longer than that of rhabdomyosarcoma for CBA, C3H, and CHI mice. In some cases the average induction time of rhabdomyosarcoma in mice with a high induction time was greater than that for spindle cell sarcoma in mice with a low average induction time. The average induction time for the CHI mice, the tumors of which were examined microscopically, was much higher than that for the general group developing tumors.

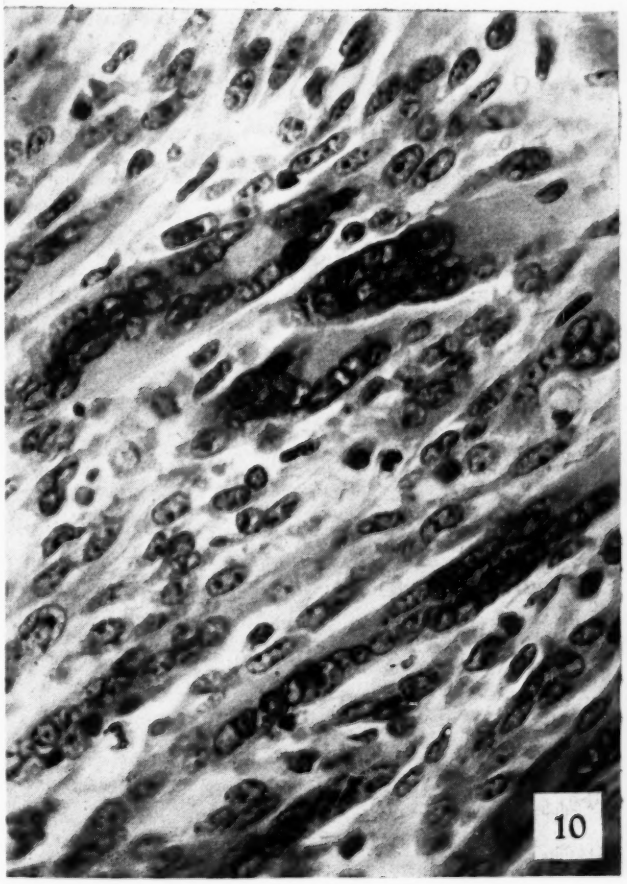
The incidence of papillomas, lung tumors, and mesenteric metastases is given in Table III. The lung tumors and mesenteric metastases are tabulated only for mice the tumors of which were sectioned. The lung tumors appeared microscopically as in Fig. 12.

TABLE II: TYPES OF TUMORS OCCURRING IN MICE INJECTED SUBCUTANEOUSLY WITH METHYLCHOLANTHRENE

	CBA			C3H			CHI			JK		
	Number	Per cent	Average induction time	Number	Per cent	Average induction time	Number	Per cent	Average induction time	Number	Per cent	Average induction time
Spindle cell sarcoma	17	55	111.3	20	26	83.6	11	34	155.0	44	65	140.2
Rhabdomyosarcoma	10	32	88.9	48	62	66.3	16	50	141.7	17	25	148.4
Epidermoid carcinoma	2	6	61.5									
Anaplastic sarcoma				1	1	88.0						
Mixed tumors:												
Carcinoma-spindle cell sarcoma				2	3	71.0						
Carcinoma-rhabdomyosarcoma				4	5	68.8				1	1	90.0
Spindle cell sarcoma-rhabdomyosarcoma	2	6	100.0	3	4	72.0	5	16	113.7	5	7	110.6
Spindle cell sarcoma-anaplastic sarcoma-rhabdomyosarcoma										1	1	184.0
Total	31			78			32			68		







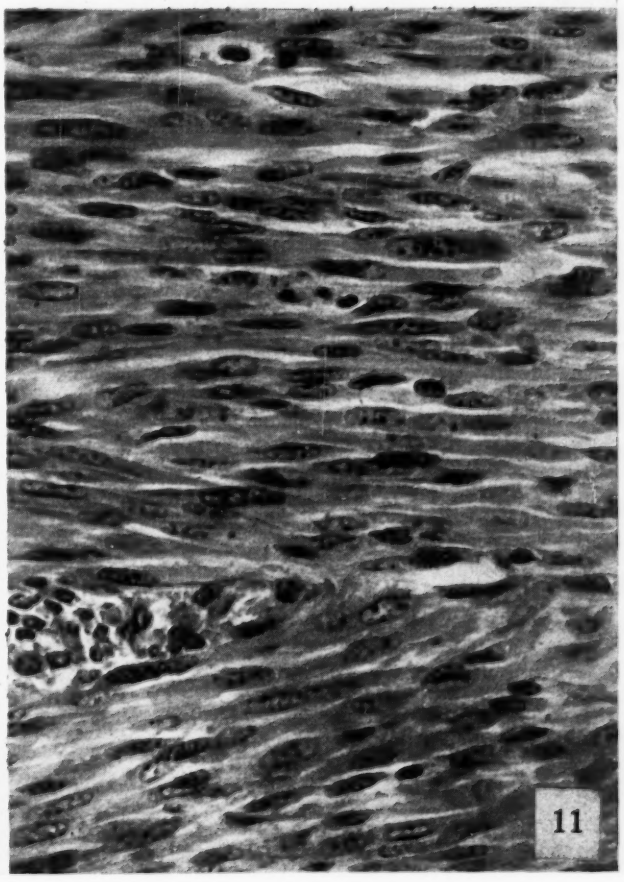
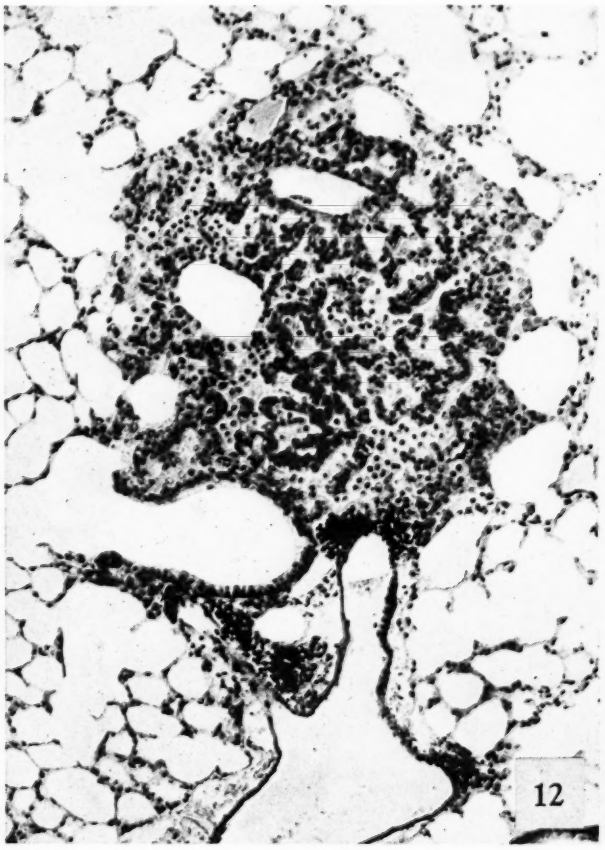


FIG. 8.—Mixed tumor: carcinoma-and-spindle cell sarcoma. Mag. × 55.
FIG. 9.—Rhabdomyosarcoma. Mag. × 400.
FIG. 10.—Rhabdomyosarcoma. Mag. × 265.
FIG. 11.—Spindle cell sarcoma. Mag. × 265.

All the tumors occurred on the right side where the carcinogen was injected. When there were mesenteric metastases there was usually direct extension of the tumor through the body wall, the mesentery, and often infiltration of the pancreas. Such an extension of a spindle cell sarcoma through the pancreas is illustrated by Fig. 13. Rhabdomyosarcomas were the only other tumors behaving in this manner. At times the mesentery was studded with small and large tumors. The lienal mesentery was so involved more often than any other.

TABLE III: NEOPLASIA OTHER THAN AT THE INJECTION SITE OF METHYLCHOLANTHRENE

	CBA		C3H		CHI		JK		NH	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Lung tumors			1			5	4	7	1	4
Mesenteric metastases			2			7				3
Papillomas					1			2	4	1



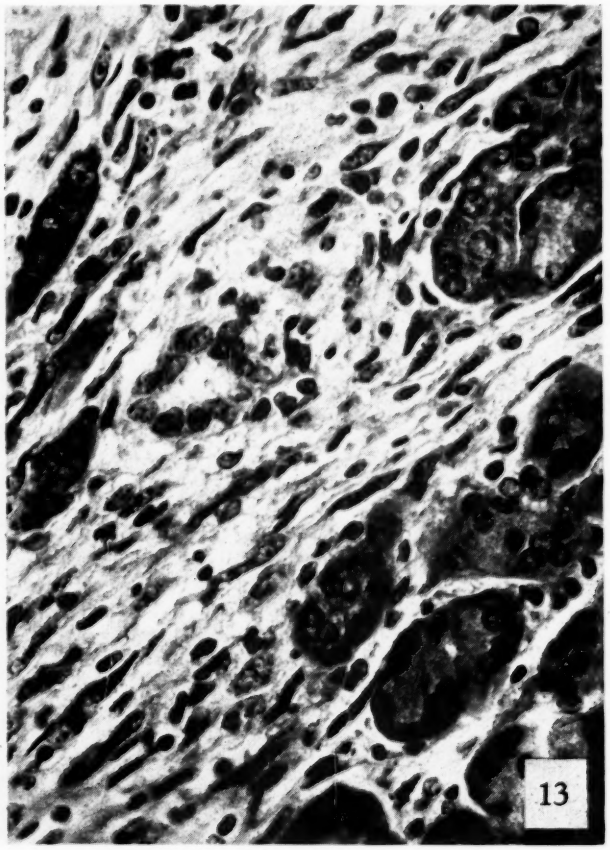


FIG. 12.—Pulmonary tumor. Mag. × 55.
FIG. 13.—Extension of a spindle cell sarcoma through the pancreas. Mag. × 265.

DISCUSSION

Differences in susceptibility to tumors induced by painting carcinogens have been found by several investigators. Using strain 3, with a high incidence of spontaneous mammary cancer, and a hybrid strain with a lower incidence of this tumor, Reinhard and Candee (12) found that strain 3 had a shorter latent period for tumors induced by painting the mice with tar than had the hybrid strain under the same treatment. Five strains of mice were tested by Lynch (11) by applying a coal tar residue to the skin, and she found a difference between the strains in susceptibility to the induction of tumors. Strain C57 showed a higher incidence of tumors than the dba strain when both were painted with tar, according to Korteweg (8). Approximately twice as many tumors developed in the skin of C57 as in A mice when Branch (4) painted them with 1,2,5,6-dibenzanthracene. Using tar paintings, Kreyberg (9) discovered a difference in the time of development of papillomas and malignant tumors in two inbred strains.

With the injection technic, also, differences in susceptibility have been demonstrated. Boyland and Warren (3) found that the appearance time of tumors in the Simpson albino strain was less than that in the CBA strain when both were injected with methylcholanthrene in lard. The carcinogen, 1,2,5,6-dibenzanthracene, was injected subcutaneously in

small doses in five inbred strains by Dobrovolskaia-Zavadskaia and her coworkers (7). These strains were free from spontaneous mammary cancer. They developed tumors in response to the carcinogen, and strains XXXIX and XLI were most susceptible. Andervont (1) injected a lard solution of 1,2,5,6-dibenzanthracene and methylcholanthrene subcutaneously in eight inbred strains. He found that the C3H strain was most susceptible to induced neoplasms and the I and Y strains were most resistant. Intermediate susceptibility was found for the C, M, C57, A, and D strains. Bonser (2) reported that in IF mice tumors developed later than in A, CBA, White Label, and market mice when they were injected with a lard solution of methylcholanthrene.

A sex difference in susceptibility to induced tumors has been reported by Reinhard and Candee (12) and Kreyberg (9). Their results showed a longer latent period before tumor formation in the males, whereas Andervont (1) reported a longer latent period in females of the C57 strain but cautioned against accepting this without reservation. Sall and Shear (13) found a higher percentage of tumors in males. Susceptibility to induced tumors paralleled that of spontaneous mammary tumors in the strains used by Reinhard and Candee (12), Boyland and Warren (3), and Bonser (2). However, the reverse relationship was true in those studied by Korteweg (8), Branch (4), and Kreyberg (9). Andervont (1) could find no constant relationship between the two in eight strains studied. Smaller doses of the carcinogen than the one used in this experiment might possibly reveal greater differences in susceptibility (13). With the larger dose, however, the time during which the experiment is in progress is considerably reduced, and the tumors occur before mammary growths and other neoplasms complicate the picture.

The curves for induction time of the three stocks, CBA, C3H, and CHI, having origin in the A × D cross, are grouped together (Fig. 7). There are differences and similarities in the susceptibility of these closely related strains to tumors induced by methylcholanthrene. The curves for the CBA and CHI strains are very similar, and the curve for the C3H strain is different from those of the CBA and CHI strains. Although originally the C3H strain was selected for early appearance of spontaneous mammary tumors and the CBA strain for longevity, there is now also a difference in susceptibility to tumors induced by methylcholanthrene. The NH strain, derived from the JK and indirectly from the CBA strain (Fig. 1), has a curve of induction time falling between those of the stocks from which it came, and the values for the induction time are nearer the stock to which it is more closely related (Fig. 7). For further genetic studies of the susceptibility under consideration, the C3H and JK mice, as the most divergent of the strains in this respect, are the logical choice.

The predominant type of tumor, rhabdomyosarcoma or spindle cell sarcoma, was not the same for all strains (Table II), and the average induction time of spindle cell sarcoma was longer than that of rhabdomyosarcoma in all except JK individuals. However, the type of tumor developing was not necessarily secondary to the induction time, since the average induction time of rhabdomyosarcoma in mice with a high induction time was greater than for the spindle cell sarcoma in mice of a low average induction time in some cases.

Although Shimkin (14) found that the survival times of C3H and Y males was not different, the survival times of the mice studied in this experiment were not always the same. Exactly the same relationship will not always hold, then, when tumor susceptibility is judged by the death of tumor-bearing animals as compared to the appearance time of the tumors. There is no indication that there is a sex difference in the susceptibility of these strains to induced tumors, with the possible exception of the CHI strain. Although the susceptibility of these strains to induced tumors falls in the same sequence as their susceptibility to spontaneous mammary tumors (16), the two are not necessarily related.

CONCLUSIONS

1. Differences in the time of appearance of tumors induced by a single injection of methylcholanthrene were detected among five inbred strains of mice.

2. The predominant type of tumor was spindle cell sarcoma in some strains and rhabdomyosarcoma in others.

3. The survival time of mice developing tumors did not parallel the susceptibility of the mice to induced tumors.

REFERENCES

1. Andervont, H. B. Susceptibility of Mice to Spontaneous, Induced, and Transplantable Tumors. A Comparative Study of Eight Strains. Pub. Health Rep., **53**:1647-1665. 1938.
2. Bonser, G. M. Induction of Tumors by Injected Methylcholanthrene in Mice of a Strain Especially Sensitive to Carcinogenic Agents Applied to Skin, and a Comparison with Some Other Strains. Am. J. Cancer, **38**:319-327. 1940.
3. Boyland, E., and Warren, F. L. The Induction of Tumors by Methylcholanthrene in Two Strains of Mice. J. Path. & Bact., **45**:171-177. 1937.
4. Branch, C. F. Dibenzanthracene Tumors in Controlled Strains of Mice. Am. J. Cancer, **26**:110-114. 1936.
5. Burdette, W. J., and Strong, L. C. The Incidence of Methylcholanthrene Induced Tumors in Inbred Strains of Mice. Genetics, **26**:143. 1941.

6. Burdette, W. J., and Strong, L. C. Comparison of Methyl Salicylate and Benzene as Solvents for Methylcholanthrene. Cancer Research, **1**:939-941. 1941.

7. Dobrovolskaia-Zavadskaia, N., and Adamova, N. Réaction à différents agents cancérigènes, de souris appartenant à des lignées exemptes d'adéno-carcinome de la mamelle. Bull. Assoc. franç. p. l'étude du cancer, **27**:308-341. 1938.

8. Korteweg, R. Experimenteel Onderzoek Aangaande de Erfelijkheid van Kanker. Nederl. Tijdschr. v. Geneesk., **77**:4038-4050. 1933.

9. Kreyberg, L. The Genetic and Constitutional Aspects of Spontaneous and Induced Tumors. In A Symposium on Cancer. Pp. 3-19. Madison: The University of Wisconsin Press. 1938.

10. Lewis, W. H. Dibenzanthracene Mouse Sarcomas. Histology. Am. J. Cancer, **37**:521-530. 1939.

11. Lynch, C. J. Strain Differences in Susceptibility to Tar-Induced Skin Tumors in Mice. Proc. Soc. Exper. Biol. & Med., **31**:215-217. 1933.

12. Reinhard, M. C., and Candee, C. F. Influence of Sex and Heredity on the Development of Tar Tumors. Am. J. Cancer, **16**:640-644. 1932.

13. Sall, R. D., and Shear, M. J. Studies in Carcinogenesis. XII. Effect of the Basic Fraction of Creosote Oil on the Production of Tumors in Mice by Chemical Carcinogens. J. Nat. Cancer Inst., **1**:45-55. 1940.

14. Shimkin, M. B. Length of Survival of Mice with Induced Subcutaneous Sarcomas. J. Nat. Cancer Inst., **1**:761-765. 1941.

15. Strong, L. C. A Genetic Analysis of the Induction of Tumors by Methylcholanthrene. With a Note on the Origin of the NH Strain of Mice. Am. J. Cancer, **39**: 347-349. 1940.

16. Strong, L. C. The Origin of Some Inbred Mice. Cancer Research, **2**:531-539. 1942.

A Genetic Analysis of the Induction of Tumors by Methylcholanthrene

IV. The Probable Remote Induction of Various Types of Gastric Lesions

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Tumors of the forestomach in mice can be produced consistently by carcinogenic chemicals (6, 2), but in the glandular portion they rarely appear. In 1941, at the Conference on Gastric Cancer (7), the discussion of experimental gastric tumors was concluded with the expressed hope that cancer of the glandular portion would ultimately be elicited. Prior to 1941 there are but four references to experimentally induced adenocarcinoma of the stomach in animals. Voronoff and Alexandrescu (12) fed white rats a mixture of tar, wool, fat, aniline oil, and toluylenediamine 3 times a week and reported the occurrence of an adenocarcinoma in one rat dying 6 months later. The tumor was described as invading the gastric wall but the illustrations do not show the deeper layers of the stomach or the invading cells. Metastases were not present. Waterman (13) reported many glandular polyps in the distal stomach of one of 6 mice fed cholesterol oleate 3 times a week for 360 days. The lesion was not described, and there was no mention of metastases.

Roffo (4) found a variety of lesions, including hyperplasias, ulcers, cysts, and polyps in rats that had been fed either irradiated cholesterol or animal fats preheated to 350° C. for half an hour. Several of the glandular ulcers were interpreted as showing malignant changes, and the more advanced tumors were regarded as adenocarcinomas. Domagk (3) described a gastric adenocarcinoma that showed local invasion in one of 20 mice fed a diet of rice and olive oil for 365 days.

Rusch, Baumann, and Maison (5) surgically exposed the stomachs of 5 rats and injected benzpyrene in oil into the wall of the glandular portion. After 15 months a low grade adenocarcinoma was found, which was described as invading the muscular tissue but without metastases.

Recently Stewart (8) reported the occurrence of adenocarcinoma of the stomach in 4 male mice of the C3H strain. At the start of the experiment each animal received an injection of 0.05 cc. of a suspension of methylcholanthrene in horse serum into the wall of the pyloric stomach. The lesions were described as submucosal nodules of columnar cells with basal nuclei, forming atypical acini *that infiltrated through the muscular wall into the peritoneum*. Mitotic figures were numerous, the stroma was scanty, and metastases were not observed.

Andervont and Stewart (1) have described an adenomatous hyperplasia of the stomach of strain I mice. These lesions occurred spontaneously in practically 100 per cent of all animals of both sexes more than 7 months old. The muscular layers were not invaded, and there were no metastases. Many attempts have been made to convert this benign lesion to a malignant one, but without any reported success as yet.

MATERIALS AND METHODS

All the mice included in this report were of the NHO strain. The origin of the NH strain has been described recently (9). Briefly stated, it was originally established as a selective group of mice following tandem crosses between the CBA, N, and JK strains. Spontaneous tumors such as carcinoma of the lung, adenocarcinoma of the mammary gland, and leukemia were occasionally discovered, and one case of adenomatous hyperplasia of the stomach at 710 days of life (male mouse 152753) has also been found. As a general rule, however, the strain must be considered very resistant to spontaneous tumors, for the percentage incidence of all types together is well under 1 per cent.

In the early generations (F_3 to F_8) following the establishment of the NH strain, groups of 60 day old mice were injected subcutaneously with 1 mgm. of methylcholanthrene dissolved in sesame oil. The first paper in this series (9) gave data bearing on the conclusions that specific types of tumors were induced and that these specific responses tended to be transmitted to the direct descendants. This result was obtained by application of the well known progeny test—*i. e.*, continuing the descendants of the mouse in each generation that conformed to type.

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Thus, from the original tandem crosses referred to above two sublines were established: the unselected NH and the selected NHO strains. Because of restricted space in the laboratory all the sublines that responded by specific tumor types to the same amount of carcinogen given subcutaneously at the same age could not be continued. Consequently, all mice receiving methylcholanthrene at 60 days of life are referred to as the NHO subline.

A partial survey of the various types of tumors obtained in approximately 2,000 mice of the NHO strain injected subcutaneously with 1 mgm. of methylcholanthrene dissolved in 0.10 cc. of sesame oil at 60 days of life has already been published (9–11). In the present paper only those mice that showed gastric lesions are discussed.

RESULTS

Gastric lesions were found at autopsy in 19 mice of the NHO strain, of which 5 were adenocarcinomas or potential adenocarcinomas,[1] distinguished from adenomas and papillomas by the presence of invading tumor cells [2] (Figs. 7 to 15). These were located at the pyloric end of the stomach and could be identified in the gross by the bulging irregular walls and the firmness of the stomach in this region. When the stomachs were split longitudinally the tumors were seen to encroach upon the lumen, and in two cases they obliterated the channel. Obstruction was evidenced by the dilatation of the proximal stomach. All the tumors extended from the pyloroduodenal junction upward for variable distances. Above the lesion the glandular mucosa appeared normal. At the pyloroduodenal junction the growths were pedunculated and extended into the duodenum. The size of the tumor was usually greater on the greater curvature of the stomach although both curvatures were marked by the growths (Figs. 1 and 3).

Microscopically the adenocarcinomas were composed of well differentiated columnar cells. The cells were arranged in small, closely packed groups or about central spaces forming acini, and were present in the submucosa, muscularis, and on the serosal surface (Figs. 2 and 12 to 15). As a result of invasion the stomach wall was usually completely disorganized (Fig. 4). The stroma of the tumors was usually scanty. The adenocarcinomas were found after an average period of treatment of 237 days.

Adenomas were found in the stomachs of 9 mice, and glandular hyperplasias in the stomachs of 4. In the gross the adenomas and glandular hyperplasias were similar to the adenocarcinomas, evident on palpation and growing into the lumen of the stomach (Fig. 5). Microscopically the adenomas were composed of hyperplastic columnar cells, heaped up and resting on folds of connective tissue (Fig. 6) with no evidence of invasion through the muscularis mucosa. Glandular hyperplasia appeared as an increase in the epithelial elements and a piling up of columnar cells without any folding of the underlying connective tissue.

A carcinoma on the greater curvature of the forestomach was found in one mouse at autopsy. This was an anaplastic, rapidly growing, squamous cell tumor composed of many cuboidal cells but also with pavement epithelium and occasional pearls. The tumor cells had invaded all layers of the stomach, disorganized the wall, and were growing on the serosal surface.

Squamous cell papillomas were found in the stomachs of 3 mice with adenomas or hyperplasia. They were located on the greater curvature and were composed of hyperplastic, well differentiated, squamous epithelium with extensive keratinization, covering either a broad or a narrow connective tissue stalk. In one instance the growth was sessile, and in the remaining two pedunculated.

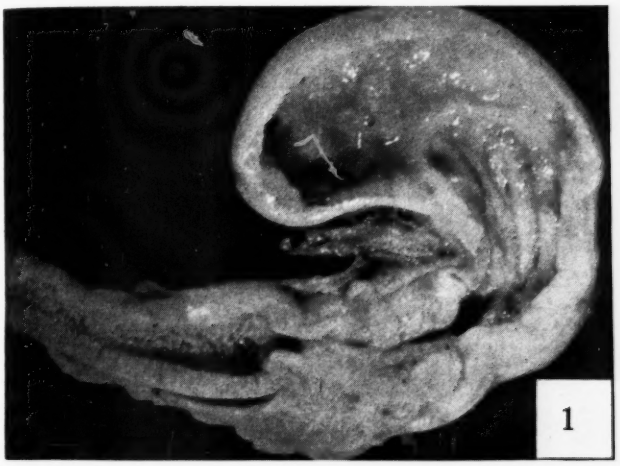
A summary of the gastric lesions found in 19 NHO mice folllowing the subcutaneous inoculation of 1

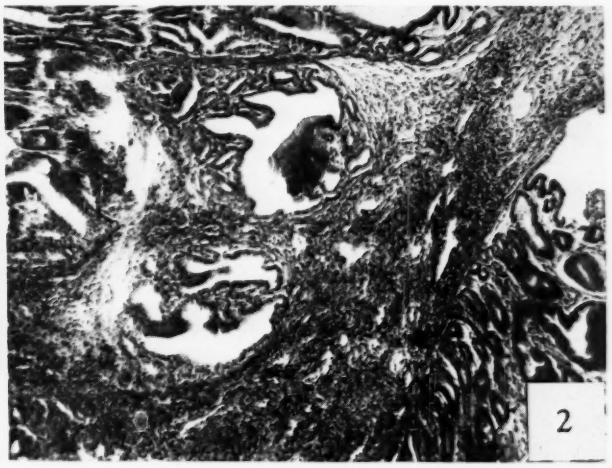
[1] The diagnosis of adenocarcinoma of the pyloric part of the stomach in mice is a difficult task. According to Kaufmann's discussion of human gastric tumors (Pathology for Students and Practitioners. Translated by S. P. Reimann. P. 694. Philadelphia: P. Blakiston's Sons & Company. 1929) "Progressive invasion of very atypical epithelial forms from the level of the mucosa into the deeper layers of the wall, eventually as far as the serosa, is one of the characteristics which distinguish carcinoma from the heterotopic gland proliferations in chronic gastritis and especially from benign glandular neoplasms or adenomas."

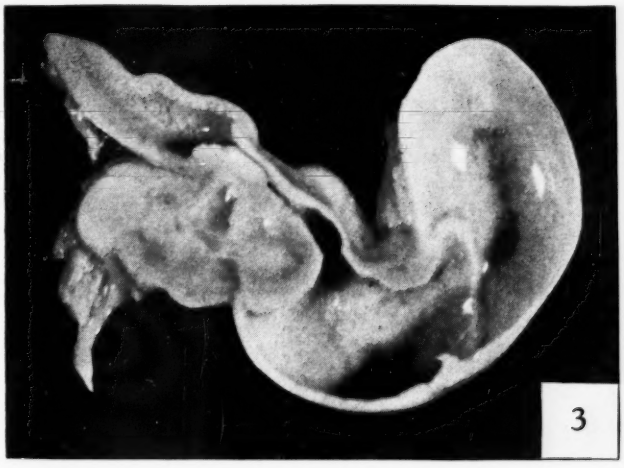
Stewart, on the other hand, states that "the only sound criterion for the diagnosis of adenocarcinoma of the glandular stomach in the mouse is that the lesion shall penetrate through all coats of the gastric wall and form a definite neoplastic nodule on the serosa."

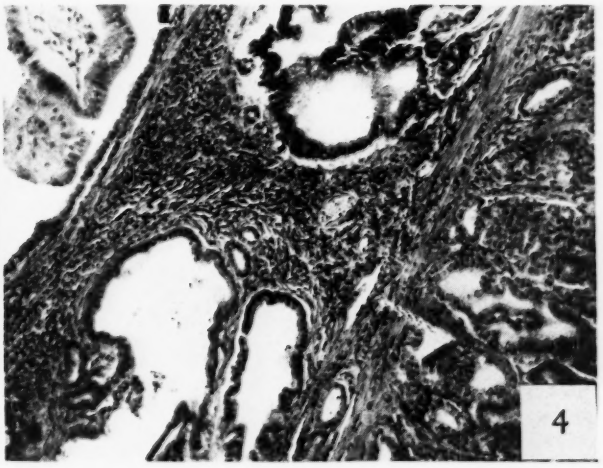
Both Shields Warren and Stewart have examined the slides of the mice tabulated as adenocarcinoma in Table I and both are agreed that invasion of tumor cells has occurred below the muscularis mucosa. Shields Warren writes "This I am forced to consider an adenocarcinoma of the gastric mucosa. The tumor is well differentiated and apparently arose in a gastric polyp. In spite of the excellent differentiation, the evidence of invasion and anaplasia are such that I feel we must put it in the group of adenocarcinomas in spite of the great rarity of this lesion in animals." Dr. Stewart reports that he has never seen glandular tissue below the muscularis mucosa in the stomach of a normal mouse, but that many old untreated mice of various strains may show adenomatous changes in the gastric glandular mucosa with ectopic glands in the submucosa. Mice of this series were certainly not old at the time the gastric lesions showing invasion were found, having been 167, 261, 466, 219, and 264 days old respectively. Stewart also states that these lesions appear to be more localized than those found in mice of strain I. In this paper the term "adenocarcinoma" or "potential adenocarcinoma" is used to distinguish those gastric lesions where some degree of invasion beyond the muscularis mucosa is evident.

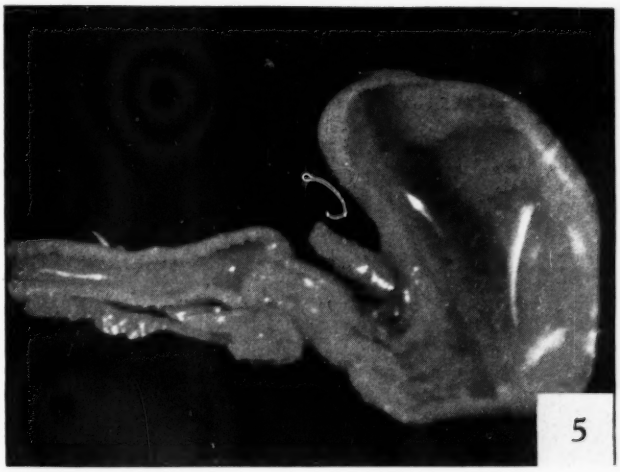
[2] These growths were examined by Dr. H. Zimmerman, of the Department of Pathology, Yale University School of Medicine, and the diagnoses substantiated.











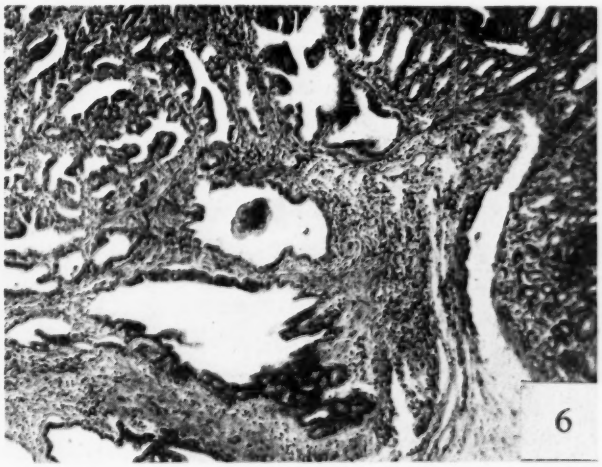


FIG. 1.—Adenocarcinoma of pyloric stomach in male mouse 164822, 143 days after subcutaneous injection of methycholanthrene. Mag. × 2.

FIG. 2.—Adenocarcinoma of Fig. 1, showing acinar formations and disorganization of the gastric walls. Mag. × 100.

FIG. 3.—Adenocarcinoma of pyloric stomach in male mouse 163414, 434 days after subcutaneous injection of methylcholanthrene. Mag. × 2.

FIG. 4.—Tumor of Fig. 3, showing columnar epithelium and acini in the serosa. Mag. × 100.

FIG. 5.—Adenoma of pyloric stomach in female mouse 163413, treated for 163 days with methylcholanthrene. Mag. × 2.

FIG. 6.—Adenoma of Fig. 5 with connective tissue base surmounted by glandular epithelium. No invasion. Mag. × 100.

mgm. of methylcholanthrene dissolved in sesame oil is given in Table I, and a more detailed tabulation in Table II.

GENERAL DISCUSSION

The appearance of gastric adenocarcinoma or potential adenocarcinoma following the subcutaneous injection of methylcholanthrene was unexpected, for it is generally held that tumors arise locally through direct effect of the carcinogen. This concept is based chiefly upon the fact that the great majority arise at the site of injection. Among the first to be obtained at remote sites were adenocarcinomas of the lung, but it was concluded that these also can be referred to direct action when the following facts are taken into consideration: The mice are known to have licked off carcinogen that had been painted on the skin, and particles of coal tar could be recovered from practically every tissue of the body, even when it had been applied on the surface.

TABLE I: SUMMARY OF 22 LESIONS OF THE STOMACH OBSERVED IN 19 NHO MICE INJECTED WITH 1 MGM. OF METHYLCHOLANTHRENE SUBCUTANEOUSLY

Type of lesion	Number of mice	Period from injection to death: Range, days	Period from injection to death: Average, days
Glandular hyperplasia	4	154–294	217
Adenoma	9	163–443	297
Adenocarcinoma	5	143–434	237
Squamous cell papilloma	3	188–443	338
Squamous cell carcinoma	1	616	616

Gastric tumors have been reported when the carcinogen has been brought into direct contact with the gastric cells by either one of two methods: through the mouth (2, 3, 4, 6, 7, 12, 13), and injected directly into the gastric mucosa (5, 8).

The present series of gastric tumors, including 5 adenocarcinomas or potential adenocarcinomas, obviously introduces a new method for the induction of these growths. For the carcinogen to have acted directly upon the gastric cells it must have entered the blood stream and become dispersed throughout the body, but why there should be a localizing or selective action upon gastric cells is not completely known. Though 7 of the 19 mice with gastric lesions also developed adenocarcinomas of the lung, many without gastric lesions also had adenocarcinomas of the lung, so it would seem that the two localizing actions (upon stomach and upon lung) are entirely independent of each other.

Where only a few rare tumors occurred in a relatively large series of mice (2,000), exceptional complications may have entered into the result obtained. Two such factors may have been: seepage of carcinogen to the surface and its ingestion as the animal cleansed itself, or perforation of the body wall by the hypodermic needle. It is felt, however, that neither of these could have been operative, and for the following reasons: In the first place, whenever the carcinogen has been administered by mouth, squamous hyperplasias and carcinomas of the forestomach have been the result, whereas in this experiment pyloric lesions were the predominant type. In the second place, when a similar amount of methylcholanthrene dissolved in sesame oil has been administered directly into the peritoneal cavity, diffuse sarcomas have been the result.

In view of the similarity of some of these gastric changes to those found in untreated mice of several strains, particularly of strain I, perhaps some further statement should be made. It is Stewart's opinion that these lesions, particularly when some invasion below the muscularis mucosa has taken place, are more localized than those observed by him and Andervont in untreated animals. In the present series the lesions have occurred in fairly young animals at relatively short times after the injection of methylcholanthrene. As those gastric lesions that showed some invasion through the muscularis occurred earlier, on the average, than adenomatous hyperplasia, it would seem that several distinct lesions must have been present in the various mice, rather than one single continuous process. The great rarity of any similar condition in control animals of this strain (only one adenomatous hyperplasia after 701 days among several hundred control animals) would probably indicate that the carcinogen may have had some influence in the initiation of these gastric lesions, although one should not be too dogmatic in this matter.

Biological variability of the mice may to a certain extent explain the diversity of tumor types obtained by the injection of NHO mice with equal amounts of methylcholanthrene at the same age. These mice belong to the first eight generations following hy-

DESCRIPTION OF FIGURES 7 TO 11

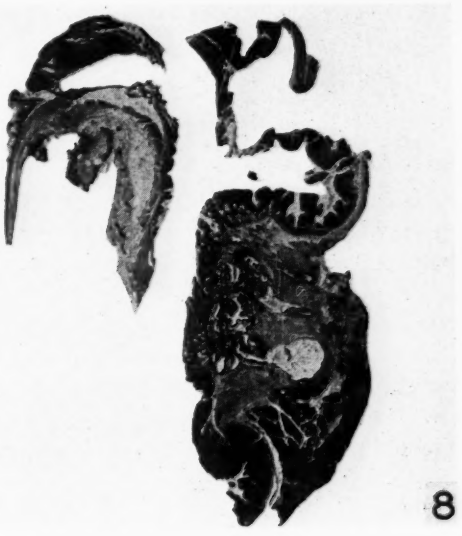
FIG. 7.—Adenocarcinoma in mouse 164822. Note nearly complete obliteration of lumen. Mag. × 5¾.

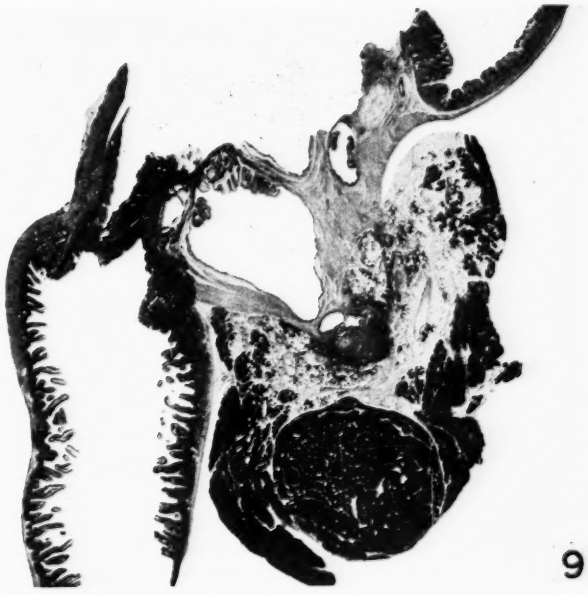
FIG. 8.—Adenocarcinoma in mouse 164821, showing invasion nearly to the pancreas. Mag. × 5¾.

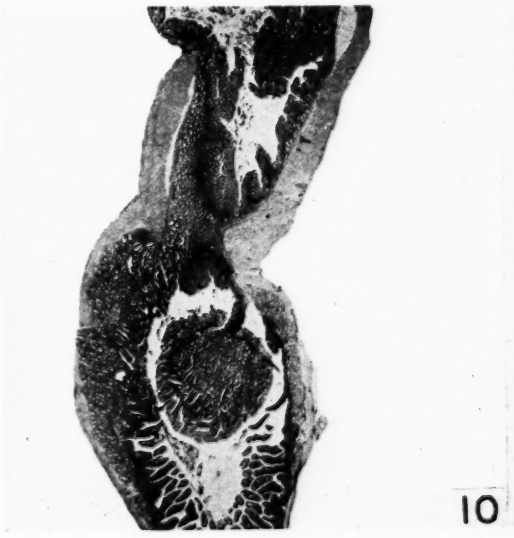
FIG. 9.—Adenocarcinoma in mouse 163414, also hyperplasia in peripancreatic lymph node. Mag. × 7.

FIG. 10.—Adenocarcinoma in mouse 162279; pedunculated duodenal polyp. Mag. × 10.

FIG. 11.—Adenocarcinoma in mouse 161439, causing nearly complete obliteration of lumen. Mag. × 5¾.







FIGS. 7-11

TABLE II: MICE THAT DEVELOPED GASTRIC LESIONS AFTER INJECTION WITH METHYLCHOLANTHRENE

Mouse No.	Sex	Period from injection to discovery of local tumor, or death, days	Location of tumors	Gastric lesions	Local lesion
152391	F	595	Gastric Lung (6 carcinomas)	Squamous cell carcinoma, forestomach	
155324	M	324	Gastric Local	Sessile adenoma, pyloric stomach	Spindle cell sarcoma
156701	M	298	Gastric Lung (1 carcinoma) Local	Two adenomatous polyps	" " "
156921	M	442	Gastric Local Lung (1 carcinoma)	Squamous cell papilloma Adenoma, pyloric stomach	" " "
158732	M	384	Gastric	Squamous cell papilloma Adenoma, pyloric stomach	
159128	M	285	Gastric	Adenomatous polyp	
161439	F	205	Gastric Local	Adenocarcinoma, pyloric stomach	" " "
162279	M	169	Gastric Local	" "	" " "
162479	M	201	Gastric	Adenoma, pyloric stomach	
162657	M	310	Gastric	Three adenomas	
162778	M	206	Gastric Local Lung (1 carcinoma)	Adenoma	" " "
163413	F	159	Gastric Local	Adenoma, pyloric stomach	" " "
163414	M	416	Gastric Local Lung (1 carcinoma)	Adenocarcinoma	" " "
164554	M	253	Gastric Local Lung (1 carcinoma)	Adenomatous hyperplasia, pyloric stomach	" " "
164817	F	173	Gastric Local	Squamous cell papilloma Glandular hyperplasia	" " "
164821	F	211	Gastric Local	Adenocarcinoma	" " "
164822	M	117	Gastric Local Lung (1 carcinoma)	"	" " "
164956	M	139	Gastric Local	Glandular hyperplasia	" " "
165041	M	213	Gastric Local	" "	" " "

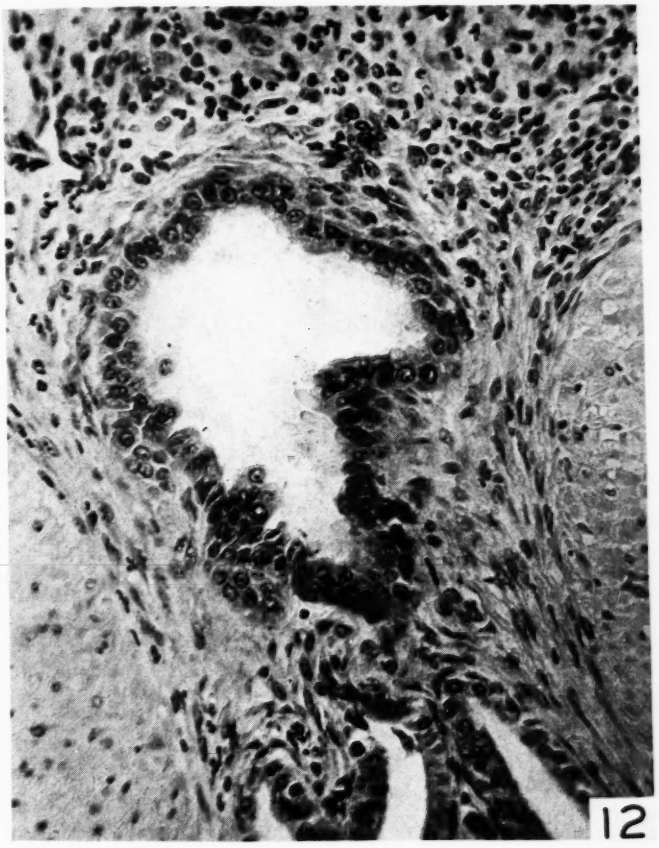
DESCRIPTION OF FIGURES 12 TO 15

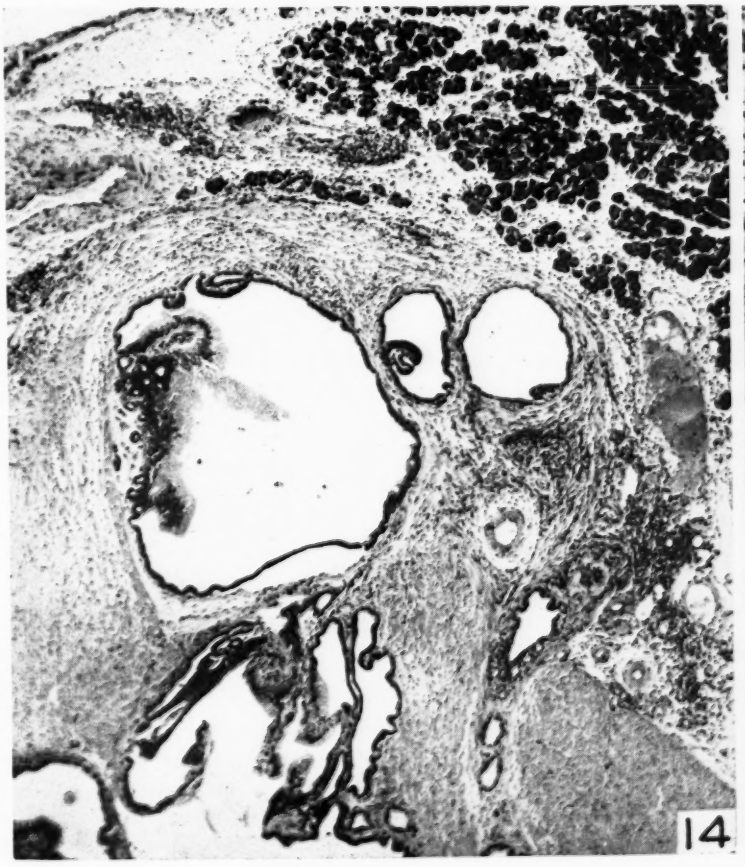
FIG. 12.—Adenocarcinoma of stomach in mouse 164821. Tumor invading through external muscle layer near base of site of inflammation. Mag. × 275.

FIG. 13.—Adenocarcinoma of stomach in mouse 164822. Invasion through external muscular layer. Mag. × 185.

FIG. 14.—Another section of tumor from mouse 164821, showing invasion through the external muscular layer and encroaching upon the pancreas. Mag. × 50.

FIG. 15.—Stomach lesion in mouse 162279 invading external muscular layer at site of inflammatory reaction. Mag. × 185.





FIGS. 12-15

bridization and do therefore possess biological variability which, when measured by specific induced tumor types, shows response to genetic selection; but it is also known that some variation in tumor types is obtained even when inbred strains of mice are used.

The present investigation on the induction of specific types of tumors by the same carcinogen administered subcutaneously to an extensive series of hybrid mice leads to the following general conclusion: In the induction of specific tumors there is a different frequency distribution for each type following a different latent period for each type of cell. The gastric hyperplasias and adenocarcinomas reported in this paper were probably induced because the gastric cells were receptive or sensitive to the "carcinogenic stimulus" at this particular time. The specific cellular constituents of the different viscera and tissues of the organism probably show chronological variations in receptivity to "carcinogenic" as well as to other stimuli; thus the aging process, as applied to cancer susceptibility, should be applied to specific cell types just as much as it is to the organism as a whole.

CONCLUSIONS

1. A variety of gastric lesions, including glandular hyperplasia, adenoma, adenocarcinoma or potential adenocarcinoma, squamous cell papilloma, and squamous cell carcinoma have been obtained in mice of the NHO strain after subcutaneous injection, at 60 days of life, of 1 mgm. of methylcholanthrene dissolved in 0.1 cc. of sesame oil.

2. It appears, although it is not definitely proved, that the presence of the carcinogen may have had some influence on the appearance of these gastric lesions.

3. These gastric lesions occurred in mice that had also developed adenocarcinoma of the lung or spindle cell sarcoma, or quite independently of any other form of neoplasia.

REFERENCES

1. Andervont, H. B., and Stewart, H. L. Adenomatous Lesion in Stomach of Strain I Mice. Science, **86**:566-567. 1937.
2. Collins, V. J., Gardner, W. U., and Strong, L. C. Experimental Gastric Tumors in Mice. Cancer Research, **3**:29-35. 1943.
3. Domagk, G. Weitere experimentelle Untersuchungen über die Ursachen des Krebses. Ztschr. f. Krebsforsch., **48**: 283-297. 1939.
4. Roffo, A. H. Bildung von Geschwüren und bösartigen Geschwülsten im Verdauungsapparat durch Einführung von Nahrung mit bestrahltem Cholesterin. Ztschr. f. Krebsforsch., **47**:473-497. 1938.
5. Rusch, H. P., Baumann, C. A., and Maison, C. L. Production of Internal Tumors with Chemical Carcinogens. Arch. Path., **29**:8-19. 1940.
6. Stewart, H. L. Induction of Gastric Tumors in Strain A Mice by Methylcholanthrene. Arch. Path., **29**:153-162. 1940.
7. Stewart, H. L. Hyperplastic and Neoplastic Lesions of the Stomach in Mice. J. Nat. Cancer Inst., **1**:489-509. 1941.
8. Stewart, H. L., and Lorenz, E. Induction of Adenocarcinoma of Pyloric Stomach in Mice by Methylcholanthrene. J. Nat. Cancer. Inst., **2**:193-196. 1941.
9. Strong, L. C. A Genetic Analysis of the Induction of Tumors by Methylcholanthrene. With a Note on the Origin of the NH Strain of Mice. Am. J. Cancer, **39**: 347-349. 1940.
10. Strong, L. C. A Genetic Analysis of the Induction of Tumors by Methylcholanthrene. II. The Influence of Spindle Cell Sarcoma and of Carcinoma of the Skin upon Each Other. Cancer Research, **1**:572-574. 1941.
11. Strong, L. C., and Williams, W. L. A Genetic Analysis of the Induction of Tumors by Methylcholanthrene. III. Local and Remote Induction of Carcinoma of the Mammary Gland. Cancer Research, **1**:886-890. 1941.
12. Voronoff, S., and Alexandrescu, G. Contribution à l'étude des tumeurs expérimentales par substances chimiques. Tumeurs abdominales multiples chez le rat blanc, expérimentalement produites par l'ingestion longtemps répétée d'un mélange à base de goudron. Neoplasmes, **8**:129-181. 1929.
13. Waterman, N. Experimental Production of Carcinoma in Stomach of Mice. Acta Cancrol., **2**:375-388. 1936.

Experimental Gastric Tumors in Mice*

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The literature on experimental gastric cancer up to 1940 has been thoroughly reviewed by Klein and Palmer (3). The conclusion reached was that "there is no well established case of an adenocarcinoma of the stomach produced experimentally. Some success has attended efforts to induce squamous cell carcinoma of the forestomach in mice and rats, although considerably less than the claims in the literature would indicate."

In 1940 Stewart (6) reported 4 squamous cell papillomas and 4 squamous cell carcinomas among 30 mice of the A strain given methylcholanthrene. The carcinogen was dissolved in liquid petrolatum and injected into the anterior wall of either the glandular stomach or the forestomach when the mice were 3 months old. Tumors were found at autopsy from 1 to 17 months later. All were of the forestomach.

Lorenz and Stewart (4) administered orally aqueous emulsions of olive oil or mineral oil solutions of 20-methylcholanthrene and 1,2,5,6-dibenzanthracene to mice of the A, C57 black, C57 brown, and C3H strains. After 7 months 24 papillomas and 10 squamous cell carcinomas were found at autopsy. The total number of animals in each strain employed is not stated, but all tumors occurred in mice of the A strain, and only after treatment with methylcholanthrene.

The first report on the use of benzpyrene in producing gastric lesions appeared in 1936 (1, 2, 5). The carcinogen in lard was fed once a week to 20 mice; after 16 months only one showed hyperplasia of the forestomach.

Waterman (9) fed benzpyrene in lard to 6 mice of unspecified strains by inserting a glass rod smeared with this material into the oral cavity. This was done daily and after 112 to 336 days 5 of the mice had tumors of the forestomach. These were described as composed of squamous cells, and 3 of the animals had metastases to the portal lymph nodes, peritoneum, and liver.

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** This material is based on a thesis by V. J. Collins submitted to the Faculty of the School of Medicine, Yale University, in candidacy for the degree of Doctor of Medicine.

MATERIALS AND METHODS

The first series of 35 ovariectomized mice of the inbred C3H strain received intravaginal instillations of 0.3 mgm. of benzpyrene [1] dissolved in sesame oil (10 mgm. of benzpyrene in 1 cc.). Twice weekly 0.03 cc. was instilled at each treatment through a blunted 22 gauge hypodermic needle. When the mice were released a part of the solution invariably escaped from the vaginal orifice and spread over the surrounding skin, whereupon it was noted that the animals would lick off the excess fluid.

When treatment started the mice were between 41 and 136 days of age, and had been ovariectomized about 1 week before the initial treatment. The period of treatment lasted from 198 to 336 days (Table I). During this time the animals were kept in wooden boxes divided into two compartments. A sawdust mat was kept on the floor of the cages and there were never more than 6 mice to a compartment. At all times food (Purina fox chow) and tap water were available to the animals.

When an animal appeared moribund or died, an autopsy was immediately performed and various tissues were fixed in Bouin's fluid.

A second series consisted of 98 mice from five inbred strains, namely C3H, A, NH, CHI, and C57. Except for a group of 26 male animals of the C3H strain, all were either spayed or intact females. A solution of benzpyrene, 10 mgm. per cc. of sesame oil, was administered orally twice a week to make a total weekly dosage of 0.6 mgm. The technic of treatment was to hold the mouse firmly in one hand and to inject 0.03 cc. of the solution into the pharynx through a blunted 22 gauge needle. The mice readily swallowed most of the material but occasionally some spread over the lips and jaw.

The ages when treatment began varied between 48 and 60 days. Some mice were ovariectomized about 1 week before it was commenced. The period of treatment for the entire series ranged from 220 to 490 days. The number of animals in each strain used, the duration of treatment, and the total amount of benzpyrene given are listed in Table I.

[1] The benzpyrene was obtained from Hoffmann-LaRoche, Inc.

Housing, food, and care of these mice were the same as in the first series. The clinical course was followed closely and weights were recorded at least once a month. Whenever a decrease in activity or in weight was apparent, or an animal was found dead, an autopsy was immediately performed and tissues were fixed in Bouin's fluid or 10 per cent formalin. Microscopic examination was subsequently carried out.

Controls.—Paralleling the conditions of the second series of mice, 12 intact female mice of the C3H strain were given 0.03 cc. of sesame oil without carcinogen biweekly. At the conclusion of the experimental work most of these animals were still alive and consequently were killed and autopsied and their stomachs studied.

Also serving as control animals are more than 25,000 mice of various inbred strains, and hybrids dying of natural causes, autopsied by Dr. L. C. Strong from whom the experimental mice were obtained. No spontaneous gastric tumors have been observed in these mice (8).[2]

The malignant tumors of the forestomach invaded the gastric walls and consisted of disorganized and more hyperplastic tissue. The malignant cells extended beyond their normal boundaries and into the lymph spaces and blood vessels and occasionally proliferated in the lymph nodes, the peritoneum, or other organs. The normal architecture of the gastric mucosa and muscularis was destroyed by tumor cells. The neoplastic elements showed many mitotic figures, hyperchromasia, and large nuclei with one or more deeply staining nucleoli.

Incidence of gastric lesions.[3]—Gastric tumors occurred following both intravaginal and oral application of benzpyrene (Table I). Squamous cell papillomas and carcinomas of the forestomach were obtained. The direct oral application of carcinogen yielded a higher number of both types of tumors than did the indirect oral (intravaginal) application. Among the 35 spayed C3H mice treated intravaginally 14 papillomas and 7 carcinomas were obtained in 21 mice, or about 40 per cent and 20 per cent respectively. Among 10 spayed C3H mice treated orally 5 papillomas and 4 carcinomas were obtained in 9 mice, or 50 per cent and 40 per cent respectively.

Of 26 C3H male mice that were treated orally 18 had papillomas and 5 had carcinomas, or about 75 per cent and 20 per cent respectively. Of 9 intact C3H female mice treated orally 7 had papillomas and 2 had carcinomas at autopsy, or about 75 per cent and 25 per cent.

Among the 11 intact C57 female mice fed the solution of benzpyrene the incidence of tumors was also rather high. In this group 9 had tumors at autopsy. Of the tumors 7 were papillomas and 2 carcinomas. Among the A and CHI mice no carcinoma

TABLE I: SUMMARY OF TUMORS OBTAINED IN FIVE STRAINS OF MICE TREATED WITH 0.3 MGM. BENZPYRENE BIWEEKLY

Strain *	Number of mice treated	Length of treatment, days	Number of skin tumors		Number of gastric tumors		Gastric hyperplasia
			Papillomas	Carcinomas	Papillomas	Carcinomas	
C3H Fx †	35	198–366	12	22	14	7	2
C3H M	26	227–359	8	18	18	5	1
C3H Fx	10	220–252	5	5	5	4	0
C3H F	9	229–344	3	4	7	2	0
NH Fx	12	278–408	7	4	2	2	3
NH F	12	255–368	9	2	3	0	0
C57 F	11	269–409	6	5	7	2	1
A Fx	9	284–350	4	2	8	0	0
CHI F	9	262–345	5	1	5	0	1
C3H Fx ‡	12	181–402	0	0	0	0	0

* Abbreviations: Fx = spayed; M = male; F = female.

† This group received benzpyrene "intravaginally"; all others received benzpyrene by direct oral route; the carcinogen was dissolved in sesame oil.

‡ Control group: received 0.03 cc. of sesame oil biweekly.

OBSERVATIONS

Lesions of the skin or stomach have been classified as benign or malignant. Benign lesions were divided into hyperplasias and papillomatous tumors. The former term included any general thickening of the epithelia beyond the normal range, with an increase in the normal cellular elements and no disorganization; the latter any localized projection of tissue which, when viewed microscopically, showed an increase in the normal epithelial elements covering either a narrow or broad stalk of connective tissue. When more than one papilloma occurred only the largest was considered for purposes of tabulation.

[2] Three squamous cell tumors of the forestomach have been observed among mice in this laboratory not treated with carcinogenic hydrocarbons (J. Nat. Cancer Inst., **1**:502-504. 1941).

[3] Approximate percentages are used and are not considered statistically significant because of the small numbers of mice involved.

of the forestomach was diagnosed. Of 9 spayed mice of the A strain 8 acquired papillomas, and of the 9 intact female mice of the CHI strain 5 had papillomas at autopsy.

The incidence of gastric papillomas and carcinomas was considerably lower in mice of the NH strain. In 12 spayed mice of this strain that were fed the oily solution of benzpyrene only 4 had tumors at death, 2 of which were carcinomas and 2 papillomas. Of the 12 intact NH female mice 3 had papillomas and none had carcinoma.

Gastric tumors or lesions were not found in the control spayed C3H mice that were fed sesame oil.

Gastric hyperplasia was found only occasionally in the mice observed.

The influence of ovariectomy and sex on the incidence of gastric tumors.—Considering the mice treated orally, it is noted that in the spayed C3H strain mice 40 per cent developed gastric carcinomas. This is in contrast to the lower incidence of 20 per cent in the intact females and in the males of this same strain. In mice of the NH strain none of the 12 intact animals developed carcinoma of the stomach while carcinomas appeared in 2 of the 12 spayed. These differences are by no means statistically significant because of the small number of animals used, but they are sufficiently suggestive to indicate the desirability of further experimentation.

Table II: Incidence of Gastric Tumors Following the Biweekly Administration of 0.3 Mgm. Benzpyrene to Mice of Several Strains for Variable Periods of Time

Strain	Number of mice	Number of tumors	Number of papillomas	Number of carcinomas	Papillomas: Range of treatment, days	Papillomas: Average treatment, days	Carcinomas: Range of treatment, days	Carcinomas: Average treatment, days
C3H Fx *	35	21	14	7	287–366	335	198–284	267
C3H M	26	23	18	5	227–359	303	240–346	313
C3H Fx	10	9	5	4	271–352	321	284–344	312
C3H F	9	9	7	2	229–344	302	321–327	324
NH Fx	12	4	2	2	321–401	361	325–375	350
NH F	12	3	3	0	307–368	329	—	—
C57 F	11	9	7	2	291–409	357	305–409	357
A Fx	9	8	8	0	284–350	333	—	—
CHI F	9	5	5	0	262–345	297	—	—
C3H Fx †	12	0	0	0				

* "Intravaginal" administration of benzpyrene; all others received benzpyrene by direct oral route.
† Control animals. Received 0.03 cc. of sesame oil biweekly.

Strain differences in the incidence of gastric tumors.—The differences in the incidence of gastric tumors, particularly gastric carcinoma, were striking among mice of the various strains which had been fed carcinogen (Table II). By far the most susceptible were those of the C3H strain. More than 90 per cent of these mice developed tumors: intact males 95 per cent, intact females 100 per cent, and spayed mice 90 per cent. Carcinomas developed in 20 per cent of the males, 20 per cent of the intact females, and in 40 per cent of the spayed mice. The only other mice with a comparable incidence were those of the C57 strain in which 87 per cent acquired tumors, 20 per cent of which were carcinomas.

The most resistant strain was the CHI, in which no carcinomas occurred. Although 50 per cent of these mice had papillomas most of the tumors were small. Likewise, none of the A strain mice had carcinomas, but the incidence of papillomas was about 88 per cent and in all cases the growths were multiple, and many were large.

The incidence of gastric tumors among mice of the NH strain differed depending on the presence or absence of the ovaries. Of the 12 spayed mice 4 had tumors, of which 2, or about 15 per cent, were carcinomas. On the other hand, the 12 intact NH mice 3 had tumors, or about 25 per cent. Although these growths were all papillomas they were rather large.

Duration of treatment preceding the detection of gastric tumors.—Mice of the C3H strain acquired gastric tumors within a shorter period after the administration of benzpyrene was begun than did those of any other strain. The average period of treatment in this strain was 306 days for males, 315 days for spayed mice, and 311 days for intact females. The average period of treatment of all mice with tumors in this strain was 310 days. Carcinomas were found after an average period of treatment of 313 days for males, 312 days for spayed mice, and 324 days for intact females.

Tumors in spayed NH mice appeared at an average of 357 days and carcinomas at an average of 350 days. Mice of the C57 strain had tumors after an average period of treatment of 363 days, and carcinomas after 357 days.

Gastric carcinomas were found in intact female mice of the NH strain and CHI strain, or in the spayed

mice of the A strain. The average period of treatment before benign tumors appeared in intact NH females was 329 days, in intact CHI females 315 days, and in spayed A mice 331 days.

Location of tumors.—The site of predilection, almost exclusively, for the development of both benign and malignant gastric tumors in this experiment was the greater curvature (Table III). For the purpose of description the lesions on the greater curvature were further classified as being in the upper portion of the forestomach, or fundus, or near the limiting ridge. All carcinomas were in the fundus. Only an occasional tumor was noted either on the lesser curvature or on the anterior or posterior wall.

Gross morphology of tumors.—The number of papillomas, either pedunculated or sessile, in the stomachs of individual mice varied greatly, the pedunculated tumors predominating. Each strain of mice showed both types.

Microscopic morphology.—Each papilloma consisted of a central core of connective tissue covered with squamous epithelium. These cores were narrow and elongated in cases designated as pedunculated and broad and flat in tumors designated as sessile (Fig. 6). In both types the surface epithelium was usually thrown into spine-like projections. The epithelium was stratified and well differentiated, mitoses were rare, and the gastric wall was not invaded or disorganized.

The carcinomas were all of the squamous cell type. The degree of differentiation varied but in each tumor basal cells, prickle cells, and flat keratinized cells could be found. Several carcinomas were rather anaplastic, being composed chiefly of cuboidal basal cells. Others showed a predominance of prickle cells with numerous whorls of keratinized epithelium. Mitotic figures were frequent and occasionally tripolar.

All carcinomas invaded and disorganized the layers

TABLE III: LOCATION OF SQUAMOUS PAPILLOMAS AND SQUAMOUS CARCINOMAS OF THE STOMACH IN MICE OF VARIOUS STRAINS RECEIVING 0.3 MGM. BENZPYRENE IN SESAME OIL BIWEEKLY

Strain	Number of mice	Number of tumors	Location of the gastric tumors			
			Greater curvature		Lesser curvature	Anterior and posterior wall
			Fundus	Ridge		
C3H Fx *	35	21	8	9	2	2
C3H M	26	23	15	7	1	0
C3H Fx	10	9	5	3	0	1
C3H F	9	9	5	3	1	0
NH Fx	12	4	4	0	0	0
NH F	12	3	1	1	0	1
C57 F	11	9	7	2	0	0
A Fx	9	8	3	4	1	0
CHI F	9	5	4	1	0	0

* "Intravaginal" administration of benzpyrene; all other groups received the benzpyrene by direct oral route.

The pedunculated tumors had small pedicles surmounted by irregularly shaped masses which varied in size from 1 to 9 mm. in diameter (Figs. 1, 2, and 5). Many of these growths were thought to be sessile but examination of the attachments revealed definite narrow stalks. Upon microscopic examination these stalks proved to be of connective tissue and covered by an extensive cellular and keratinized epithelium that gave them a cauliflower appearance.

Sessile papillomas, broad with irregular surfaces and no freely movable pedicles, varied in diameter from 2 to 8 mm.

The carcinomas were usually massive, measuring from 5 × 8 × 10 mm. to 6 × 12 × 17 mm. (Fig. 3). The serosal surfaces of the stomachs were usually irregular and nodular, and frequently the lymphatics were definitely enlarged. When the organ was split open it was found that the tumors filled the upper portion and encroached upon the glandular stomach. In many cases the esophageal opening was blocked completely.

composing the gastric wall (Figs. 4 and 6). In many areas the wall was completely replaced by tumor cells while in others a discrete but frank infiltration of the muscular and serosal layers occurred (Fig. 6). Cell nests were found frequently in the lymph spaces and in 3 mice tumor cells had invaded the blood vessels of the submucosa.

The manner of infiltration of the gastric wall followed a definite pattern, especially in the muscularis where the cells proliferated along the sheaths of the muscular fasciculi (Figs. 2 and 6). Wherever tumor cells penetrated into the gastric wall an inflammatory process developed, as evidenced by the presence of polymorphonuclear leucocytes and plasma cells. Inflammatory cells were likewise present throughout the stroma of the tumors.

In the largest growths areas of softening and necrosis were frequently observed.

Skin tumors.—Squamous cell tumors of the skin developed in a very high percentage of animals (Table IV), appearing early in the course of treatment at sites

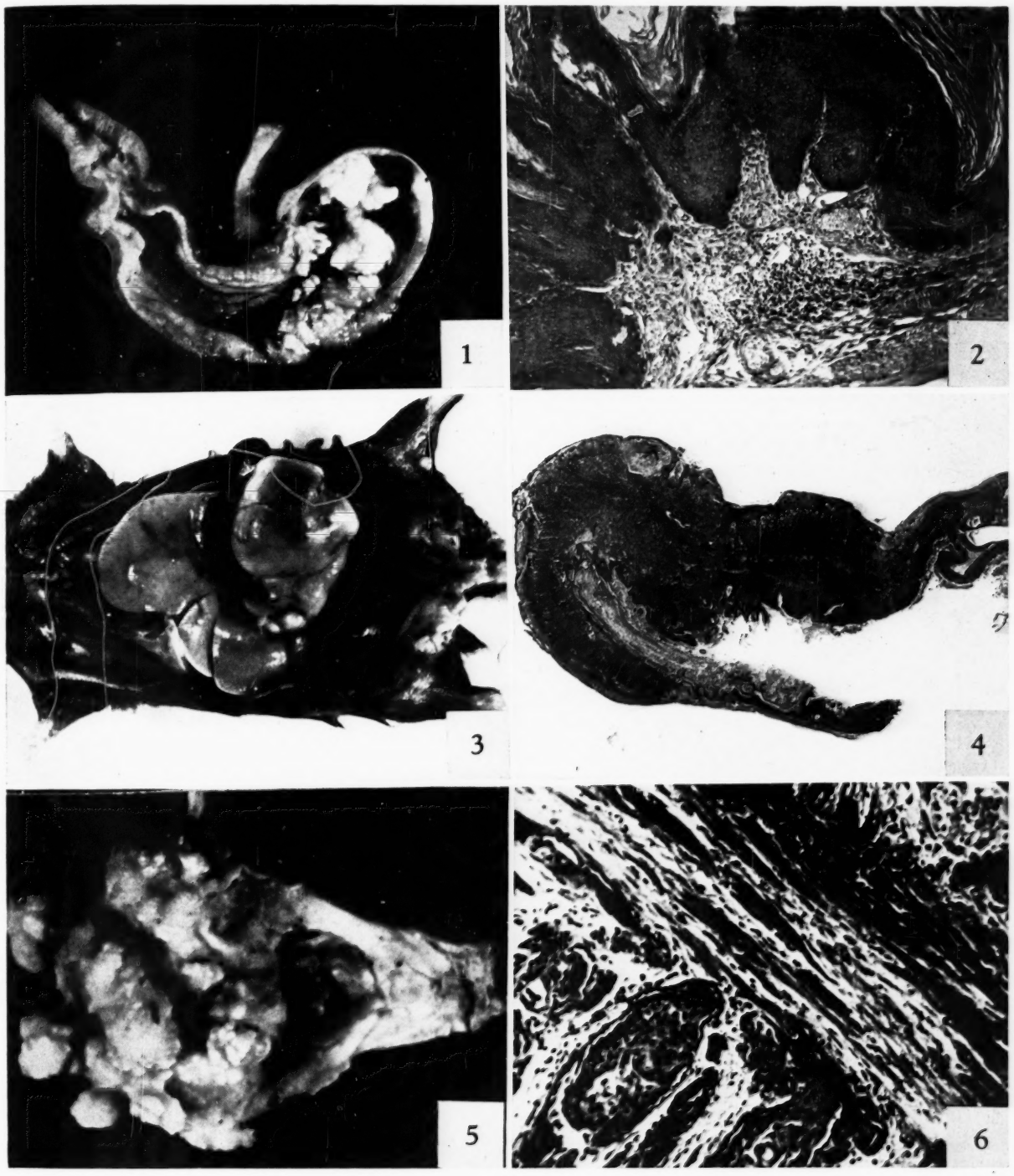

FIG. 1.—One-half the stomach of a mouse of the A strain which had received benzpyrene orally for 285 days. A large sessile papilloma firmly attached to the greater curvature and posterior wall. Mag. × 3.5.

FIG. 2.—A small sessile papilloma appearing on the greater curvature of the stomach of a spayed mouse of the C3H strain that had received intravaginal instillations of benzpyrene for 287 days. Mag. × 100.

FIG. 3.—Gastric carcinoma in a spayed C3H mouse that had received 0.3 mgm. of benzpyrene intravaginally twice weekly for 280 days. The large tumor was palpated through the body wall.

FIG. 4.—Squamous cell carcinoma of the stomach in a spayed mouse of the C3H strain that had received benzpyrene for 198 days. The tumor had invaded the submucosa and muscularis and was growing in the serosal lymphatic channels. Mag. × 9.

FIG. 5.—Multiple papillomas, largely of the pedunculated type, in the stomach of a mouse of the A strain that had received benzpyrene orally for 350 days. These tumors showed no evidence of invasion of the submucosa in the sections prepared. Mag. × 3.5.

FIG. 6.—Section of the gastric submucosa and muscularis of a spayed mouse of the C3H strain that received benzpyrene orally for 299 days. Cancer cells had invaded both the muscularis and the serosa. Mag. × 150.

contaminated by the application of benzpyrene. In the first series of mice skin lesions developed about the vaginal orifice and in the second series about the mouth. Nearly all mice of the C3H, NH, and C57 strains developed skin tumors, the majority of which were carcinomas. In mice of the A and CHI strains, on the contrary, fewer tumors appeared, and the number of carcinomas was definitely small. Susceptibility to skin and gastric tumors thus paralleled each other in this investigation.

There were 4 instances of carcinoma of the skin and carcinoma of the stomach in the same animal. In the 18 other mice with carcinoma of the stomach the skin lesions were benign and usually quite small. On the other hand, the 63 mice with carcinoma of the skin usually had small gastric papillomas and only 4 had gastric carcinomas (Table IV).

TABLE IV: TABULATION OF GASTRIC CARCINOMAS AND PAPILLOMAS IN MICE WITH SKIN CARCINOMAS

Strain of mice	Number of skin carcinomas	Number of gastric carcinomas in mice with skin carcinomas	Number of gastric papillomas in mice with skin carcinomas	Number of mice without gastric tumors and with skin carcinomas
C3H Fx *	22	3	10	9
C3H M	18	0	15	3
C3H Fx	5	0	4	1
C3H F	4	0	4	—
NH Fx	4	1	1	2
NH F	2	0	1	1
C57 F	5	0	4	1
A Fx	2	0	2	—
CHI F	1	0	1	—

* This group of mice received benzpyrene "intravaginally"; all others received benzpyrene by direct oral route; the carcinogen was dissolved in sesame oil.

Transplantation.—One carcinoma occurring in a C57 mouse was transplanted into 3 adult female mice of the same strain. Grafts about 3 × 2 mm. placed subcutaneously in the axilla when they were still growing at the end of 4 weeks, measured between 5 × 8 and 7 × 8 mm. each.

General observations.—Animals treated orally were observed twice a week during the experimental period. Weight records, made every 4 weeks, showed that the mice gained 1 to 7 gm. during the first few months of treatment and then maintained this weight during most of the experiment. During the last week or two of their lives many lost as much as 10 gm.

DISCUSSION

Tumors of the forestomach can be produced successfully and consistently in mice by the administration of benzpyrene. Consequently, it is felt that a method is available for the experimental study of the characteristics of such neoplasms. A few of these characteristics have been indicated by the experimental results.

First, there are definite strain differences in the incidence of gastric lesions among mice subjected to comparable treatment. Mice of the C3H, NH, and C57 strains are probably more susceptible than those of the A and CHI strains. With regard to the epidermal tumors induced by benzpyrene these strains show a similar distribution in their susceptibility. Thus C3H, NH, and C57 mice are highly susceptible, while the A and CHI strains are resistant to the development of squamous tumors of the skin of the contaminated perioral region. The manifestations of susceptibility included shorter latent period, higher incidence of tumors, and a greater percentage of malignancy.

Mice of the C3H and A strains are highly susceptible to adenocarcinoma of the mammary gland in contrast to those of the CHI, NH, and C57 strains, which acquire mammary tumors either very late in life or very rarely. Strain A mice, although susceptible to spontaneous mammary carcinoma, showed no squamous carcinomas of the stomach after ingestion of benzpyrene. The susceptibility to gastric tumors subsequent to the oral application of benzpyrene was not associated with the susceptibility to spontaneous mammary tumors. Mice of the A strain were the only ones in which Stewart first reported the occurrence of gastric carcinomas induced by methylcholanthrene (6). However, this difference in response might be explained by the carcinogens or solvents used, by differences in environmental factors, or of responses among different lines of the A strain.

No distinct sex difference in the incidence of gastric lesions was noted, the percentage of gastric papillomas and of gastric carcinomas having been nearly the same in males and females of the C3H strain. Nevertheless, the incidence of malignant gastric lesions in ovariectomized mice of the C3H and NH strains was twice as high as in the intact female mice of these same strains.

The small tumors were located chiefly on the greater curvature of the forestomach, and apparently most of the carcinomas arose in this area. The gastric tumors of the greater curvature appeared twice as frequently in the fundus as at the limiting-ridge areas. The reason for this is speculative but probably depends on the fact that the upper part of the forestomach is more relaxed while areas near the ridge are under the influence of pyloric motility. The carcinogenic agent would be, therefore, in contact with the epithelium of the fundus for a longer period.

The presence of one primary tumor probably influenced the development of a second. In the benzpyrene-treated animals, skin tumors appeared in a high percentage of all mice and were observed quite early.

In mice that developed large skin carcinomas the incidence of gastric malignancy was low. On the other hand, in mice developing gastric carcinomas the incidence of skin carcinomas was low and the tumors were small and benign.

The vehicle in which a carcinogen is suspended or dissolved may alter its carcinogenic action. Stewart (7) found definite differences in the incidence of gastric carcinoma when methylcholanthrene was suspended in different oils and fed to mice of the A strain. Feeding an olive oil emulsion of methylcholanthrene resulted in no gastric tumors although when this same emulsion was stabilized with a wetting agent and fed, a few tumors appeared. Feeding a mineral oil emulsion resulted in several carcinomas of the stomach. In the present investigation the carcinogen was dissolved in sesame oil and tumors were produced in some mice of the strains used.

SUMMARY AND CONCLUSIONS

1. Benzpyrene dissolved in sesame oil was administered to 133 mice in five inbred strains, namely C3H, NH, C57, A, and CHI, to determine its effect upon the formation of tumors of the gastrointestinal tract.

2. All tumors involving the stomach apparently arose in the forestomach and were of the squamous cell type. The highest incidence of gastric papillomas and carcinomas occurred in mice of the C3H strain. A high incidence of gastric tumors was observed in mice of the C57 and NH strains and a low incidence of carcinomas in mice of the CHI and A strains.

3. No definite sex difference in the incidence of gastric tumors was noted between males and females of the C3H strain. However, the incidence of carcinomas was higher in ovariectomized mice of the C3H and NH strains than in intact mice similarly treated.

4. Most gastric tumors appeared at the greater curvature of the forestomach, the fundus of which was more frequently involved than the area near the limiting ridge. Tumors occasionally developed on the lesser curvature and on the anterior or posterior wall.

5. Carcinomas and papillomas appeared in 22 and 69 of the treated mice respectively. They were of the squamous cell type, showing a mixture of keratinized flat cells, prickle cells, and basal cells.

6. The 22 carcinomas infiltrated the gastric wall, extended to the serosa, and invaded the lymphatics.

7. Papillomas and carcinomas of the skin occurred at the sites contaminated by the benzpyrene in nearly all animals. Whenever a carcinoma of the skin was found the gastric tumor was usually small and benign; on the other hand, mice with carcinoma of the stomach usually had benign skin lesions.

8. Gastric carcinoma from a C57 mouse has been successfully transplanted to 3 normal mice of the same strain.

REFERENCES

1. Cook, J. W., Haslewood, G. A. D., Hewett, C. L., Hieger, I., Kennaway, E. L., and Mayneord, W. V. Chemical Compounds as Carcinogenic Agents. Am. J. Cancer, **29**: 219-259. 1937.
2. Cook, J. W., and Kennaway, E. L., Chemical Compounds as Carcinogenic Agents. First Supplementary Report: Literature of 1937. Am. J. Cancer, **33**:50-97. 1938.
3. Klein, A. J., and Palmer, W. L. Experimental Gastric Carcinoma. A Critical Review with Comments on the Criteria of Induced Malignancy. Arch. Path., **29**: 814-844. 1940.
4. Lorenz, E., and Stewart, H. L. Squamous Cell Carcinoma and Other Lesions of the Forestomach in Mice, Following Oral Administration of 20-Methylcholanthrene and 1,2,5,6-Dibenzanthracene. J. Nat. Cancer Inst., **1**:273-276. 1940.
5. Oberling, C., Sannié, C., Guérin, M., and Guérin, P. Recherches sur l'action cancérigène du 1,2-benzopyrène. Bull. Assoc. franç. p. l'étude du cancer, **25**:156-180. 1936.
6. Stewart, H. L. Induction of Gastric Tumors in Strain A Mice by Methylcholanthrene. Arch. Path., **29**:153-162. 1940.
7. Stewart, H. L. Hyperplastic and Neoplastic Lesions of the Stomach in Mice. J. Nat. Cancer Inst., **1**:489-502. 1941.
8. Strong, L. C. Unpublished data. 1942.
9. Waterman, N. Production of Carcinoma of Stomach in Mice by 3,4-Benzpyrene. Acta. brev. Neerland., **7**:18-20. 1937.

Skin Carcinogenesis by a Single Application of 20-Methylcholanthrene*

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INTRODUCTION

In our study of the early stages of carcinogenesis (2, 3), it was found that a single external application of the carcinogen to the skin with one brush stroke was sufficient to elicit, after 3 or 4 weeks and in a considerable fraction of the animals, an epithelial hyperplasia sufficiently advanced to merit the term "precancerous." Most of the animals used in that investigation were killed within 1 month after the application, but 6 mice were allowed to survive. Of these, 3 died in the 3rd month; of the remaining 3 mice, 1 developed a carcinoma at the site of application of the carcinogen after 22 weeks and the other 2 died at about the same time without either a papilloma or a carcinoma. At that time the only observations recording carcinogenesis in the skin by a single application of a carcinogen were those of Mider and Morton (12, 13) on mice belonging to the C57 brown strain after a single application of 20-methylcholanthrene. The same procedure applied by Mider and Morton to mice of the C57 black strain failed to elicit carcinogenesis. Since then Law (7) has recorded the development of carcinomas in the skin of mice of the C57 brown strain in response to a single application of another carcinogen, 9,10-dimethyl-1,2-benzanthracene. These observations will be discussed in detail later.

The present paper records the results obtained in a second experimental series of mice of the Swiss strain the skin of which was exposed to a single application of methylcholanthrene. The fact that positive results had been obtained in this second series was briefly mentioned in our previous papers, published before this experiment was completed.

EXPERIMENTAL PROCEDURE

Methylcholanthrene in 0.6 per cent solution in benzene was applied to the unepilated skin of mice of the Swiss strain with a No. 4 camel's hair brush. With the technic described in a previous paper (2), a single brush stroke delivers about 0.1 mgm. to an area of skin extending from the nape of the neck to the middle of the back. Two groups of Swiss mice were used. One consisted of 14 females about 2½ to 3 months old obtained directly from Tumblebrook Farms. These received 3 brush strokes simultaneously, the total dose delivered being therefore about 0.3 mgm. The second group consisted of 11 mice, 5 males and 6 females, bred in the laboratory from Swiss mice previously subjected to a treatment with methylcholanthrene over a short period of time, so that only a fraction of the animals developed skin cancer and were killed. The surviving negative mice were used for breeding, the offspring representing our "lab-bred" Swiss mice. These lab-bred Swiss mice were given only 1 brush stroke, thus receiving each about 0.1 mgm. of the carcinogen. This second group gave a negative result, while in the first mentioned group, which had received 0.3 mgm. of the carcinogen, 6 mice developed malignant tumors. It cannot, however, be concluded that this difference between the two groups is entirely due to the difference in dosage of the carcinogen; because, as already mentioned, the first carcinoma obtained by us in the Swiss strain, in the course of our studies on the early skin changes after a single application of methylcholanthrene, resulted from a single brush stroke. The lab-bred mice of the second group were derived from parents that had been relatively resistant to the action of the carcinogen. Hence it may be argued that the lab-bred mice represented a resistant substrain of the Swiss strain and that the failure to elicit carcinogenesis by a single application was due to this factor alone or to a combination of this factor and diminution in dosage.

The following account, therefore, refers exclusively to the 14 female mice belonging to the first group, all of which were alive at the time the first malignant tumor appeared (experiment XL). The data on the 6 mice that developed malignant tumors are given in Table I.

In 5 of the 6 mice with malignant neoplasms, the tumor developed in the skin area exposed to the direct action of the carcinogen. In one mouse (No. 1), the tumor, a sarcoma, was situated behind the right ear;

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i. e., just outside the area of the brush stroke but within the area to which the solution had spread by capillarity. In each of the animals the neoplasm appeared as a single tumor. Five of the tumors were carcinomas; one (in No. 3) was associated with a sarcoma.

The process of carcinogenesis differs in some significant respects from that induced by the frequent application of carcinogens, which is the method generally in use. After these multiple treatments carcinomas develop frequently, though by no means always, in association with a heavily keratinized papilloma, which precedes the development of the carcinoma. The papilloma increases in size over a considerable period of time without undergoing a malignant change until eventually a carcinoma develops either at its base or from its side. In our experience, most of the carcinomas induced by the frequent application of methylcholanthrene at short intervals have tumor in appearance. Both developed as shallow ulcers which extended progressively. There are, therefore, indications that the type of carcinoma induced in the skin by the carcinogen is dependent to a certain extent upon its mode of application, in the sense that those developing after a single application are on the average more anaplastic, more malignant, and less heavily keratinized than those occurring after frequently repeated applications over long periods of time.

Comparison with Observations of Other Workers

Mider and Morton (12) were the first to record the development of carcinomas in the skin of mice of the C57 brown strain after a single application of methylcholanthrene. They painted a large area of skin with a 0.5 per cent solution of methylcholanthrene; thus their experimental conditions were similar to ours. In their first series, 3 of 44 mice developed carcinomas in a period of induction varying from 16 to 32 weeks. In their second, carcinomas developed in 7 of 156 mice in a similar period of induction, varying from 15 to 33 weeks (13). In 28 mice of another strain, the C57 black, the same technic failed to induce any carcinomas.

Table I: Carcinogenesis in Swiss Mice after a Dosage of 0.3 Mgm. Methylcholanthrene, Delivered in 3 Brush Strokes

Mouse No.	Period of induction, weeks	Type of neoplasm	Histologic type	Macroscopic appearance
1	13	Sarcoma	Spindle cell	Tumor ulcerating through skin
2	15	Carcinoma	Squamous carcinoma, anaplastic	Tumor
3	18	1 carcinoma 1 sarcoma	Squamous carcinoma mixed with spindle cell sarcoma	Large ulcer with high rolling edge
4	21	Carcinoma	Anaplastic carcinoma with lymphatic permeation	Shallow ulcer
5	25	Carcinoma	Squamous carcinoma	Shallow ulcer
6	42	Carcinoma	Squamous carcinoma	Shallow ulcer

been squamous cell carcinomas of varying degrees of malignancy. So far as we know this has also been the experience of other workers. Many of these squamous carcinomas have been of a low type of malignancy; *i. e.,* composed of typical squamous cells forming a heavily keratinized solid neoplasm. Basal cell carcinomas develop rarely, if at all, in the mouse.

In the 6 mice belonging to the series reported here only 1 of the squamous carcinomas (in No. 2) can be described as having developed from a papilloma. The other 4 originated in massively hyperplastic epidermal epithelium and appeared macroscopically as ulcers which extended progressively. They showed a high degree of malignancy, the cells being anaplastic in mice 2 and 4, while in mice 3 and 5 small isolated groups of cells were scattered widely through the dermis. The tumor in No. 4 was a very anaplastic carcinoma with extensive lymphatic permeation, in which the cells had a resemblance to the basal cell tumors of man (Fig. 1). The carcinoma that developed in the one surviving animal of the preliminary experimental mice was almost identical with this

Law (7), using 9,10-dimethyl-1,2-benzanthracene and applying it in a 0.3 per cent solution to a large area of the skin of mice of the same C57 brown strain used by Mider and Morton, also succeeded in inducing carcinomas. But his results differed in several important aspects both from those of Mider and Morton and from ours. No less than 8 of 10 mice developed carcinomas, the period of induction was longer (32 to 60 weeks), and in only 2 of the mice did the carcinomas develop within the painted area of skin. Most of the animals had multiple carcinomas outside the painted area, some of the tumors developing even on the legs or on the ventral aspect. Law's results may be due to the fact that the carcinogen used in

his experiments had a decidedly toxic effect, as he pointed out. That this may induce a general systemic change in addition to the local effect on the skin is indicated by his statement that "the majority of mice surviving the toxic effect of the carcinogen developed a leukemoid condition."

Our own results with mice of the Swiss strain correspond closely to those of Mider and Morton. The length of the induction period is similar, but there is a much higher percentage of carcinogenesis in the Swiss strain. The possibility of inducing the development of a carcinoma by a single application of a

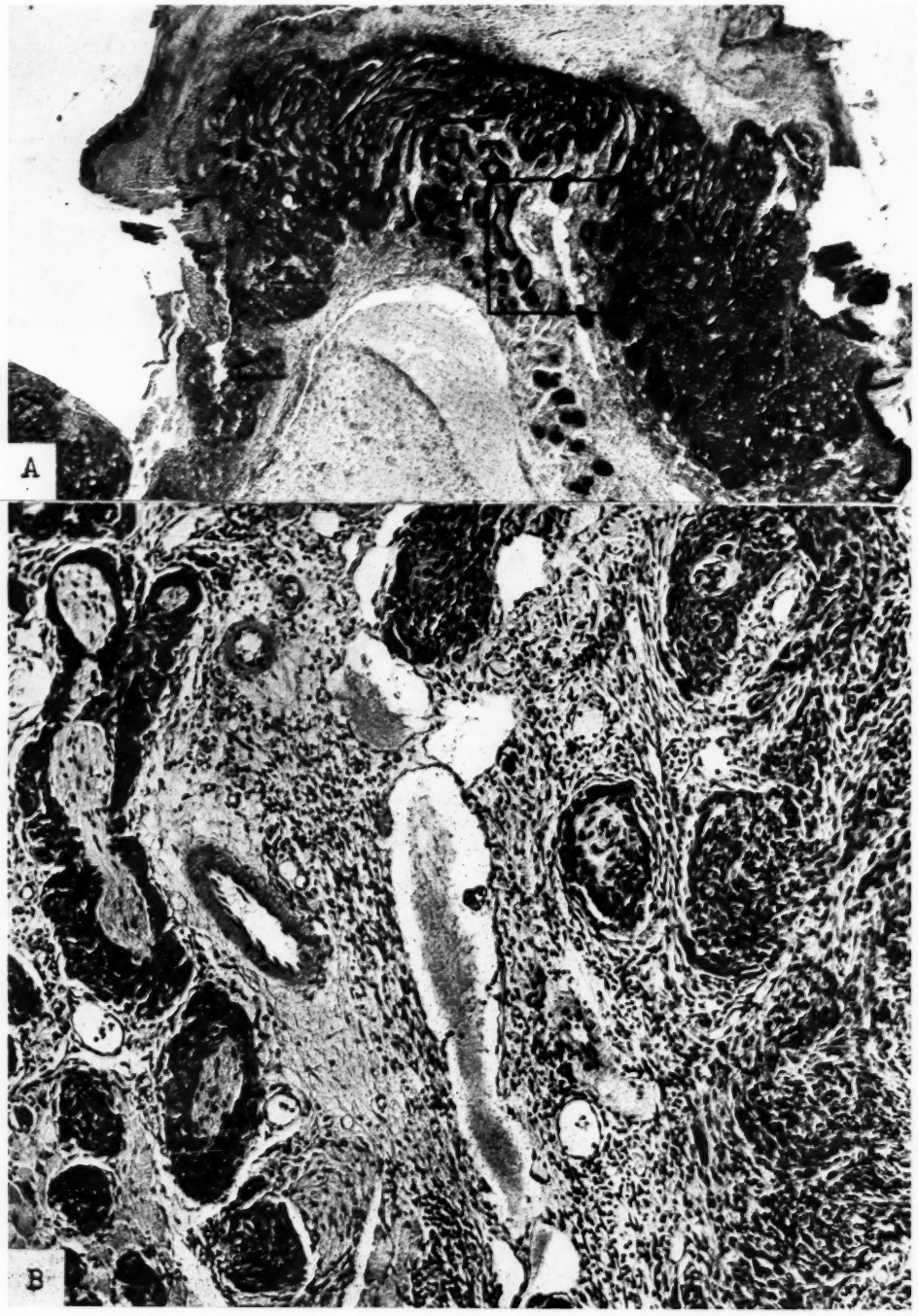

FIG. 1.—Experiment XL, Mouse 4. Ulcerating anaplastic carcinoma which appeared 22 weeks after a single application of methylcholanthrene. Bouin fixation. *A* is a low power view of the tumor. Mag. × 18; *B* shows the area outlined in *A* under a higher power. Mag. × 110. On the right side of the figure is the lower margin of the growth; on the left is a cutaneous nerve infiltrated by cancer cells; below it are round groups of cancer cells lying in lymphatics. This permeation of lymphatics extends widely through the dermis as seen in *A* below the outlined area.

potent carcinogen is, therefore, not restricted to one particular strain. The further fact that in our experiments, as in those of Mider and Morton, the neoplasms developed in the area of skin exposed to the carcinogen makes it unnecessary to postulate a general systemic effect as a contributing factor in the process of carcinogenesis by methylcholanthrene.

Relation of Single Application of the Carcinogen to a Short Exposure

Consideration must be given to the question: Does a single application signify a short exposure to the carcinogen? When a carcinogen is injected subcutaneously and a sarcoma develops, there has been a single application of the carcinogen but there has also been a continuous exposure of the tissue to it, since the carcinogen persists at the site of injection in demonstrable amounts. The same consideration applies to the development of carcinomas in the ears of sheep branded with hot tar as observed in Australia, or in the skin of mice treated with a drop of hot tar (6). In these two conditions the hot tar produces a burn. In the process of healing the tar becomes enclosed and microscopically demonstrable amounts of it remain in the skin for a long period. This mode of single application represents, therefore, an intracutaneous application with a continuous exposure to demonstrable amounts of the carcinogen. It differs essentially from the single extracutaneous application in which cancer develops in the absence of demonstrable amounts of the carcinogen. It is generally accepted that under this condition the carcinogen is rapidly absorbed by the skin and that its presence either on the surface or within the skin cannot be demonstrated after a short interval, variously estimated to be a matter of a few days or of 2 weeks.

Mode of Action of Methylcholanthrene

Until recently it has been considered essential for the development of malignancy to have the tissue subjected to an unremitting exposure to the carcinogen. Thus Fieser (5) stated in 1938 that "in order to produce skin tumors in mice with even a highly potent carcinogen in benzene solution some 30 deliberate applications must be made." The universal practice in experimental carcinogenesis, of submitting the skin to applications of a carcinogen repeated at very short intervals and continued for several months, was based on the assumption that the carcinogen elicits epithelial proliferation by maintaining, over a long period, a direct, stimulating effect on the cells. This conception appeared to be supported by the fact that with this technic the carcinogenic effect increased with increasing concentrations of the carcinogen in the solvent. From this point of view, it is interesting to compare the carcinogenic effect of a single extracutaneous application involving a short exposure with the carcinogenic effect of extracutaneous applications repeated 3 times weekly over periods of 2 and 4 weeks, respectively, as observed in two additional experiments on 20 female Swiss mice in each group. The differences in the number of applications, the total amounts of methylcholanthrene applied, and the length of time over which the skin was exposed to the action of the carcinogen are given in Table II. In this table the dose of carcinogen delivered at each brush stroke is taken to be 0.1 mgm., and it must be remembered that in the experiment with a single application 3 brush strokes were made at the same time, while in the other two experiments only 1 brush stroke was given at each application. The time of exposure is calculated on the assumption that the bulk of the carcinogen has disappeared from the skin after 1 week. If the continued exposure of the skin to the carcinogen is as essential as is generally supposed, the carcinogenic effect should be signally increased with an increase in both the dose and the time of exposure to the carcinogen.

Table II

Number of applications	Total amount of methylcholanthrene applied to skin, mgm.	Time of continued exposure, weeks
1	0.3	1
6	0.6	3
12	1.2	5

Table III: Percentages of Mice with Methylcholanthrene-Induced Malignant Skin Tumors

Month of experiment	After 1 painting only	After 6 paintings in 2 weeks	After 12 paintings in 4 weeks
1	0	0	0
2	0	0	0
3	7	10	6
4	14	16	19
5	28	21	19
6	36	21	37
7	36	26	43
8	36	42	50
9	36	42	50
10	43	47	50
11	43	47	50

The results, summarized in Table III, show that in all three series the first malignant neoplasm appears at the same time, *i. e.* in the 3rd month after the beginning of painting, and that the subsequent development of malignant tumors in the three series is almost identical until the 6th month. It is only in the later stages of the experiment that the percentage of tumors is slightly higher in the series in which the application of the carcinogen was continued for

4 weeks. But even in this series 50 per cent of the mice remained free from cancer, indicating that the individual mice resistant to a single application of the carcinogen retain their resistance even after 12 applications repeated every second day for 4 weeks.

We again draw attention to the fact, recorded in a previous paper (4), that a single application of methylcholanthrene will produce a massive hyperplasia within 4 weeks in some mice (Fig. 2), while in others applications repeated 3 times weekly for 2 months or more will fail to do so. It is possible, therefore, to distinguish even in mice belonging to the same strain two distinct groups: (a) animals which react to a single application of the carcinogen with the production of a massive hyperplasia which may or may not lead to cancer—the "susceptible group"; and (b) animals in which the skin, though suffering the initial injury inflicted by a single application of the carcinogen, merely regenerates without developing subsequently a massive hyperplasia—the "resistant group." The experiments just mentioned show that the resistant group fails to react even to an application of the carcinogen continued over 4 weeks. The existence of such differences of susceptibility and resistance in the process of carcinogenesis in the skin was recognized in the early experimental work on tar cancer, in which the carcinogenic agent was much less potent than methylcholanthrene. But we can now identify the vague terms "resistance" and "susceptibility," which may be assumed to be reciprocal, with the visible reaction to a single application of a potent carcinogen. The questions now arise whether the factors determining susceptibility are inherited and fixed in the individual organism, or whether they are capable of being varied. We already know that the resistance of the skin can be broken down by the application of a potent carcinogen over a sufficiently long period, especially if a large area of skin is exposed to its action. In this prolonged application the carcinogen is more effective, if efficiency is measured by the dose necessary to induce cancer, when it is applied at long intervals of time than when applied unremittingly at intervals of 2 or 3 days (4). With less potent carcinogens, however, the resistant animals remain free from cancer even after a prolonged application to the skin. But we have as yet no information whether the reverse process of making a susceptible skin resistant can also be induced. The

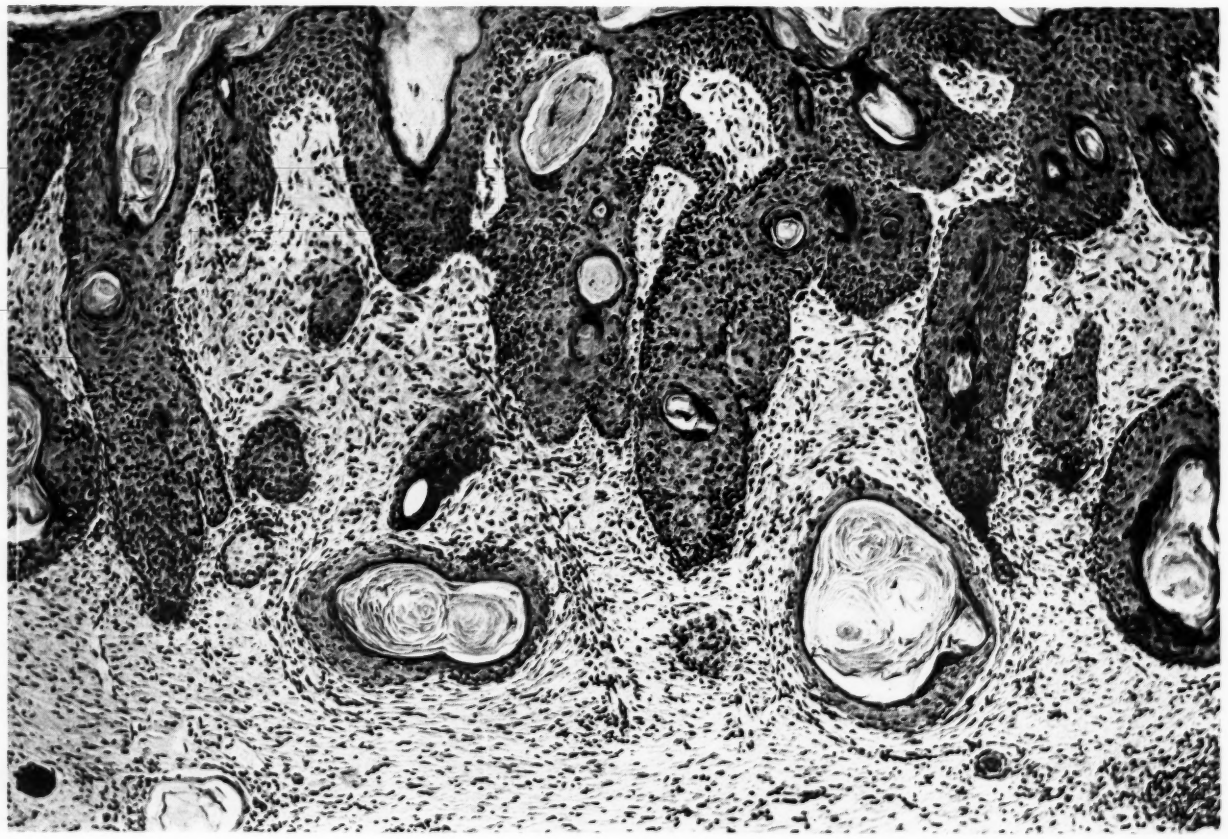

FIG. 2.—Experiment XII, 23B. Massive hyperplasia of epidermis 26 days after a single application of methylcholanthrene. Bouin fixation. Mag. × 95.

method of carcinogenesis by a single application of a potent carcinogen puts at our disposal an experimental approach to this important problem.

These considerations have a bearing on the etiology of skin cancer in man. In man it is probably rare for the skin to be exposed to a more or less continuous succession of potent carcinogenic stimuli, corresponding to an application of a highly effective carcinogen such as methylcholanthrene, extending over a period of several years. The accidental exposure over several years of the early workers with roentgen rays and radium rays was probably an analogous condition. But the majority of skin cancers in man may be assumed to be restricted to persons belonging to the group with a skin susceptible to carcinogenic agents.

In the light of the results presented in this and in previous papers, the concept that carcinogenesis depends on prolonged exposure to a carcinogen that directly stimulates epithelial proliferation requires revision. Our previous findings show that the first application of methylcholanthrene, when this is used in concentrations eliciting an optimal carcinogenic effect by the standard technic, produces injury to the epithelial layer of the skin, including the hair follicles, and not a direct stimulating effect. This injury elicits an epithelial regeneration. The regenerated epithelium is altered in its reaction to subsequent applications of the carcinogen, in the sense of having become more resistant to the toxic effects of the carcinogen. Chemical investigations on the regenerated epithelium, carried out in this hospital by Drs. L. F. Wicks, C. Carruthers, and V. Suntzeff as another part of a group investigation directed by Dr. E. V. Cowdry, show that the alteration in biologic behavior is accompanied by significant and specific chemical changes in the epidermal epithelium (1, 14). The experiments in this paper show that in a considerable fraction of the animals the regenerated epithelium proceeds, without any further intervention and in the absence of the carcinogen in amounts demonstrable by methods available at present, to the development of a massive epithelial hyperplasia which forms the basis for the subsequent development of cancer.

This change in the reaction of the epidermal epithelium to a toxic chemical substance, after an injury has been inflicted by a first application of this chemical substance, is a biologic phenomenon for which an analogy can be found in the observations of MacNider (8-11) on the epithelium of the kidney and liver that has regenerated after the administration of uranium salts and other toxic agents. But the subsequent excessive epithelial proliferation of the regenerated epithelium without any further intervention is a biologic phenomenon for which there is no analogy.

It may be argued that while the bulk of the carcinogen applied extracutaneously disappears a very small amount, not readily demonstrable by the methods available at present, remains and is responsible for the stimulating effect on the epithelium. It is possible, on general biologic conceptions, that a substance which has a toxic effect in large doses may have a stimulating effect in small doses. This possibility cannot be excluded at present. But it must be remembered that the carcinogenic effect of any chemical carcinogen tested so far by continued application diminishes with diminution of the dose applied to the skin. This means that any stimulating effect which may be attributed to very small doses of a carcinogen could become effective only on epithelial cells which have regenerated after a previous specific injury has been inflicted on the epithelium by a more massive dose.

CONCLUSIONS

Cancer can arise in the skin of mice in response to a single exposure of the skin to a potent carcinogen, such as methylcholanthrene. From this result and our previous observations, as well as from those of Mider and Morton, it appears that the conditions required to produce this response are a considerable total dose of a potent carcinogen applied to a large area of skin. Under such conditions the carcinogen injures the epithelial elements of the skin, which respond by regeneration. This regenerated epithelium is altered in its reaction to subsequent applications of the carcinogen, having become more resistant to its toxic effects. In a fraction of the animals the regenerated epithelium proceeds without further applications of the carcinogen to a massive epithelial hyperplasia culminating in the localized development of a skin carcinoma. The reaction of the skin to the carcinogen in this group of animals, the susceptible group, contrasts strongly with that of the resistant group. In resistant animals the carcinogen also produces an injury of the skin followed by regeneration. But in this group the regenerated epithelium does not proceed automatically to a massive epithelial hyperplasia; it even fails to do so when subjected to an unremitting application of the carcinogen extending over several weeks. The bearing of these observations on our conceptions of the mode of action of chemical carcinogens is discussed.

REFERENCES

1. Carruthers, C., and Suntzeff, V. Influence of Limited Application of Methylcholanthrene upon Epidermal Iron and Ascorbic Acid. J. Nat. Cancer Inst., **3**:217-220. 1942.

2. Cramer, W., and Stowell, R. E. The Early Stages of Carcinogenesis by 20-Methylcholanthrene in the Skin of the Mouse. I. Experimental Technique and Macroscopic Changes. J. Nat. Cancer Inst., **2**:369-378. 1942.

3. Cramer, W., and Stowell, R. E. The Early Stages of Carcinogenesis by 20-Methylcholanthrene in the Skin of the Mouse. II. Microscopic Tissue Changes. J. Nat. Cancer Inst., **2**:379-402. 1942.

4. Cramer, W., and Stowell, R. E. Carcinogenesis in the Mouse's Skin by the Infrequent Application at Long Intervals of Methylcholanthrene. Cancer Research, **1**: 849-852. 1941.

5. Fieser, L. F. Carcinogenic Activity, Structure, and Chemical Reactivity of Polynuclear Aromatic Hydrocarbons. Am. J. Cancer, **34**:37-124. 1938.

6. Findlay, G. M. The Experimental Production of Cancer by One Application of Tar. Lancet, **1**:714-715. 1925.

7. Law, L. W. Multiple Skin Tumors in Mice Following a Single Painting with 9:10 Dimethyl-1:2 Benzanthracene. Am. J. Path., **17**:827-831. 1941.

8. MacNider, W. de B. A Study of the Acquired Resistance of Fixed Tissue Cells Morphologically Altered through Processes of Repair. Liver Injury Induced by Uranium Nitrate. Consideration of Type of Epithelial Repair Which Imparts to Liver Resistance against Subsequent Uranium Intoxications. J. Pharmacol. & Exper. Therap., **56**:359-372. 1936.

9. MacNider, W. de B. A Study of the Acquired Resistance of Fixed Tissue Cells Morphologically Altered through Processes of Repair. Resistance of Liver Epithelium Altered Morphologically as a Result of Injury from Uranium, Followed by Repair, to Hepatoxic Action of Chloroform. J. Pharmacol. & Exper. Therap., **56**:373-381. 1936.

10. MacNider, W. de B. A Study of the Acquired Resistance of Fixed Tissue Cells Morphologically Altered through Processes of Repair. Resistance to Chloroform of Naturally Acquired Atypical Type of Liver Epithelium Occurring in Senile Animals. J. Pharmacol. & Exper. Therap., **56**:383-387. 1936.

11. MacNider, W. de B. A Study of the Acquired Resistance of Fixed Tissue Cells Morphologically Altered through Processes of Repair. Concerning the Persistence of Acquired Type of Atypical Liver Cell with Observations on the Resistance of Such Cells to the Toxic Action of Chloroform. J. Pharmacol. & Exper. Therap., **59**:393-400. 1937.

12. Mider, G. B., and Morton, J. J. Skin Tumors Following a Single Application of Methylcholanthrene in C57 Brown Mice. Am. J. Path., **15**:299-302. 1939.

13. Mider, G. B., and Morton, J. J. Relative Importance of Local and Constitutional Effects of Methylcholanthrene in Production of Skin Tumors in the Mouse. J. Nat. Cancer Inst., **1**:41-44. 1940.

14. Wicks, L. F., and Suntzeff, V. Reduction of Total Lipid-Protein Nitrogen Ratio of Mouse Epidermis by a Single Application of Methylcholanthrene. J. Nat. Cancer Inst., **3**:221-226. 1942.

Carcinogenesis after Multiple Irritation

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Although occasional clinical evidence supports the idea that the development of cancer may be facilitated by the additive effect of diverse irritations, experimental results do not confirm this view consistently. A number of experimenters, using for the most part combined irritation by carcinogenic hydrocarbons and various physical irritants, have failed to observe evidence of such an effect. Among these are Daels; Derom; Ludford; Roussy, Leroux, and Peyre; Burrows; Dietrich; des Ligneris; Parodi; and Brunschwig, Tschetter, and Bissel. On the other hand the contrary effect, of apparently increased stimulation of carcinogenesis, has been reported by Deelman; Reding; Teutschlaender; Truffi; Cramer; Raposo; Choldin; Rondoni and Corbellini; and Orr.

In the present work a study was made of the combined action of a carcinogenic hydrocarbon, 1,2,5,6-dibenzanthracene, and repeated light cauterization. To eliminate as thoroughly as possible differences attributable to individual and strain, a fairly large number of animals was used, derived from two strains of mice; the one, C57 black, with a low rate of spontaneous tumor incidence; the other, C3H brown, with a high incidence of spontaneous mammary cancers in the females. In this work, only male animals were used. All were kept in similar surroundings and on identical diets of Purina dog chow occasionally supplemented with bread; food was given in excess of actual consumption.

EXPERIMENTS

The experimental technic was as follows: The hair was clipped from the interscapular region with small scissors every 4 to 6 weeks, depending on the rate of its growth. This was retarded in the later stages of the experiment. To the clipped area 0.4 per cent solution of dibenzanthracene in benzol was applied twice weekly. In addition, half of the animals of each strain were cauterized in this area weekly by means of a flattened loop of nichrome wire, electrically heated just short of a red glow and applied lightly in such a manner as to give a linear burn about 10 mm. long. Care was taken to cauterize a fresh area of skin each time, in order to avoid multiple burning of the same region.

The weights of the experimental animals were observed weekly; after the first 3 to 5 weeks, during which the cauterization was followed by slight transient loss of weight, no differences were observable between the cauterized and control animals. The death rate from intercurrent disease was minimal; of 195 mice with which the experiment was begun 3 died during the first 120 days. Only the survivors after this period were considered in the evaluation of results—91 mice of the C3H strain, 101 of the C57. Among the latter, however, in one cage containing 13 C57 mice the animals were underweight for a period of 4 to 5 weeks, and tumor development in these was retarded beyond the usual period for this strain by from 3 to 4 weeks; these animals have been omitted from the tables and charts showing the time of tumor appearance, but included in the tabulation of total tumors.

After papillomas had appeared care was taken not to apply the cautery to them. Cauterization was discontinued after 30 weeks, or upon the first appearance of malignant growth. The application of dibenzanthracene was discontinued after 36 weeks. Individual records were kept for each animal, of the time of appearance of the first papillomas, the first definite evidence of malignancy, and the size and site of lesions initially and at death. Tumor-bearing animals were allowed to live until they became emaciated or until the tumors reached a considerable size; surviving animals were killed after 300 days. All tumors were studied histologically. The recognition of beginning malignancy was necessarily made on a purely subjective basis, by the palpation of definite marginal induration. We believe, however, that this was rather accurate, as in only two or three instances was it necessary to revise the verdict on the grounds of subsequent behavior.

RESULTS

In a control group of cauterized but unpainted animals, the following changes were noted on histological study: The immediate effect of the cauterization was destruction of the epidermis, down to the dermis and including some of the taller dermal papillae. During the next 3 days the dermis became

edematous, and there was necrosis of damaged epithelial cells and moderate leucocytic infiltration into the superficial portion of the dermis. The edema gradually subsided in the following 10 days, with accompanying diminution of extravasated leucocytes and of necrotic epithelium. During this stage evidence of epithelial regeneration appeared, with the formation of long, irregular cords of swollen cells penetrating downward into the dermis, not only in the narrow path of the burn but for a few millimeters on both sides. There was also excessive superficial epithelization, with a piling up of surface cells to a depth of 14 or more, as compared with an original thickness of from 4 to 6 layers. After from 10 to 20 days the epithelial projections shortened or disappeared, and after 3 weeks there was no evidence of the previous injury except slight epidermal thickening and very slight dermal fibrosis.

Tumor induction in the several groups of animals, under the conditions of the experiment, is most readily presented in tabular form (Table I).

TABLE I: TIME OF TUMOR INDUCTION

	C57		C3H	
	Uncauterized, days	Cauterized, days	Uncauterized, days	Cauterized, days
Period for first tumors.	130	80	150	150
Period for tumors in 50% of animals....	180	160	260	240
Period for first malignant tumor	130	120	180	180
Period for malignant tumors in 50% of animals	210	190	300	270

In these experiments the effect of cauterization was evidently the acceleration of tumor development by the dibenzanthracene irritation, as can be seen more clearly in Figs. 1 to 4. This effect is somewhat more striking with the C57 strain, but appears in both.

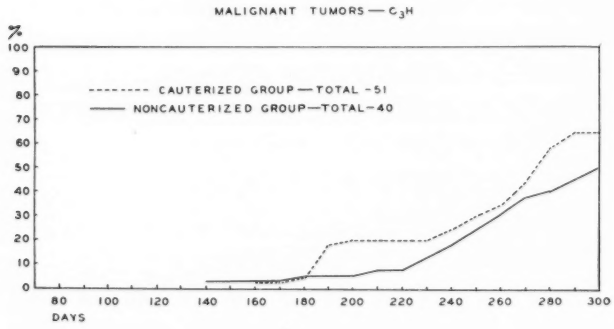

FIG. 1

Histological examination (see Table III) showed squamous cell carcinomas in 82 per cent of the cauterized animals of the C57 strain and similar tumors in 90 per cent of the uncauterized animals of this strain. In both groups 6 per cent of the animals developed sarcomas, and rare adenocarcinomas raised the total animals with malignant tumors in this strain to 90 per cent and 92 per cent respectively. There was one carcinosarcoma in a mouse of the uncauterized group.

With the C3H strain, results were definitely at variance with those for the C57 strain (Table III). While

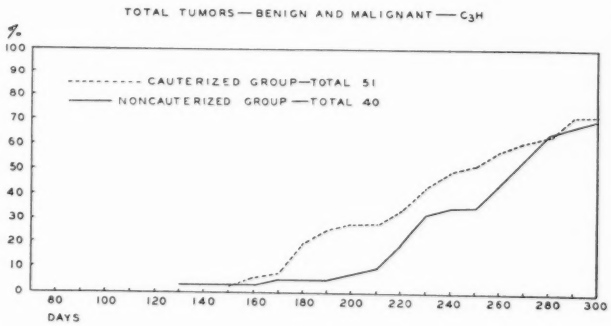

FIG. 2

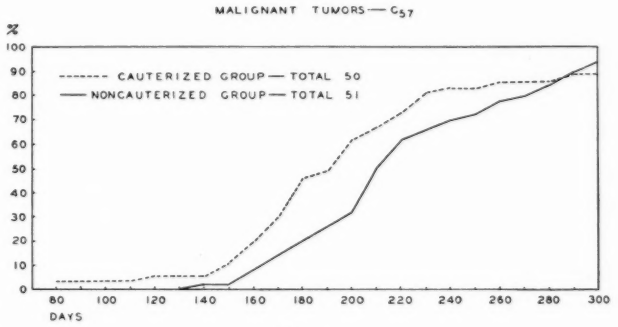

FIG. 3

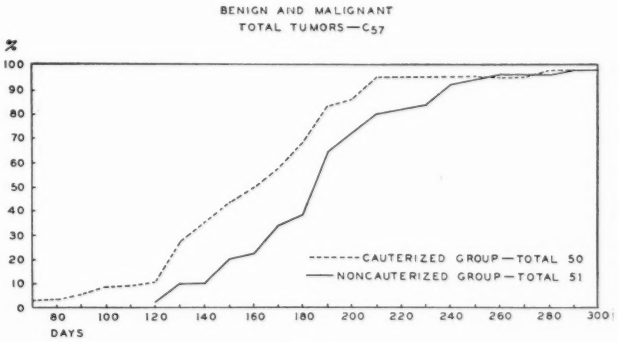

FIG. 4

the percentages of mice with squamous cell carcinomas were closely similar in the cauterized and uncauterized groups, 48 per cent and 42.5 per cent respectively, the incidence of sarcomas was much higher, reaching 26 per cent of the cauterized animals and 10 per cent of the uncauterized. In addition, 2 animals of the uncauterized and 4 of the cauterized groups developed both carcinomas and sarcomas among which is included one carcinosarcoma. Attempts at evaluating the tumors for the degree of malignancy did not show

any significant differences with respect to cauterization; but the carcinomas of the C3H strain had a somewhat higher average rating than did those of the C57 strain (Table II). As a general rule, the carcinomas of the skin in both C57 and C3H animals developed at sites of old cauterization.

TABLE II: MALIGNANCY OF SQUAMOUS CELL CARCINOMAS

	Grade			
	I	II	III	IV
C57 uncauterized	21	21	3	0
C57 cauterized	30	9	2	0
C57 total	51	30	5	0
C3H uncauterized	10	6	2	0
C3H cauterized	10	8	6	0
C3H total	20	14	8	0

Aside from the acceleration of tumor development incident to cauterization, the total occurrence of tumors in the two groups of C57 animals was about the same and was almost complete in the 300 days of observation. With the C3H animals, a wide divergence of total malignant tumors in the two groups resulted from the greater frequency of sarcomas in the cauterized animals. It would appear that the close approximation of total tumor occurrence in the C57 animals was due to the fact that the time period was sufficient to allow maximum incidence in both groups. With the C3H strain, on the other hand, this result had not nearly been reached in the same time period.

Among the workers cited in the earlier part of this article, irritation by heat was used in various forms by Daels; Parodi; Brunschwig, Tschetter, and Bissel; des Ligneris; and Cramer. Actual cauterization was used by Daels, Parodi, Brunschwig and his coworkers, and Cramer, while relatively slight heating was used by Derom and by des Ligneris. In the present experiments, while actual cauterization was practised, care was taken to limit this in order to minimize subsequent cicatrization. While it would seem difficult to explain the discrepancies in the results hitherto reported, it is possible that the element of cicatrization may have been an important factor. The acceleration observed in this work could be due to any of several factors or their combination. The accessory agent, heating, was of a type and probably of a degree sufficient to result in the formation of pyrogenic decomposition products; the entry of carcinogenic agents would be facilitated by the epithelial disintegration; and regeneration of injured areas would provide an added cause of cellular reproduction. The character of these experiments was such as to permit only inferential evaluation of the relative importance of these several factors. The second of these, readier access of the carcinogenic hydrocarbon through injured integument, would seem to be the major factor in the excessive occurrence of sarcomas in the doubly treated mice of the C3H strain. On the other hand, the fact that the skin cancers usually developed at sites cauterized some time previously, would suggest that the regenerative factor was of limited importance, if any, since regeneration in these areas would appear to have been completed long before.

With respect to the periods necessary for tumor genesis in the C57 and C3H strains, *a priori* it might be expected that malignant tumors would occur earlier in strains of animals with a high incidence of spontaneous tumors, since it might be presumed that a constitutional factor was already present there. Andervont found that sarcomas following the injection of dibenzanthracene develop more readily in such animals, while Dittmar reported that fewer tumors were induced by benzpyrene in animals of high spontaneous incidence—moderately so for skin cancers after painting, strikingly so for sarcomas after injection. Either results such as his tend to negate the theory of a constitutional element, or accessory factors mask its presence. The results obtained in this work would indicate that the constitutional factor is thoroughly masked as regards the skin in the C3H strain but that it does operate, in that the incidence of deep tumors, sarcomas, was distinctly higher in these mice. In other words, this strain seems to be more susceptible to the development of malignant tumors once the epithelial barrier is passed, as was previously found by Andervont. The much higher incidence of sarcomas in the cauterized than in the uncauterized animals of this strain also supports the view of a cutane-

TABLE III: MALIGNANT TUMORS AFTER 300 DAYS

	C57				C3H			
	Uncauterized,		Cauterized,		Uncauterized,		Cauterized,	
	No.	per cent	No.	per cent	No.	per cent	No.	per cent
Squamous cell carcinoma	45	90	41	82	17	42.5	24	48
Sarcoma	3	6	3	6	4	10	13	26
Adenocarcinoma	0		1	2	0		1	2
Both squamous cell carcinoma and sarcoma	1	2	0		2	5	4	8
Total with malignant change	47	92	45	90	20	50	34	66
Total without malignant change	4	8	5	10	20	50	17	33

ous barrier, which itself is comparatively insusceptible to carcinogenic stimulation. In the work of Dittmar the relatively massive dosage and its direct administration could conceivably have obscured parallel results.

SUMMARY

In an approximately equal number of animals of two strains, half of which were subjected to superficial application of a carcinogenic hydrocarbon and half to this procedure plus repeated light cauterization, tumors developed more rapidly in the cauterized animals.

This effect was shown more decidedly by animals of a strain with a low incidence of spontaneous cancers.

On the other hand, a considerably higher incidence of sarcomas was seen in the strain with a high incidence of spontaneous tumors, and most strikingly so in animals of this group which had been cauterized.

It is suggested that in these animals the higher incidence of sarcomas may be an expression of a constitutional predisposition already present, which is masked in the skin by some accessory factor.

REFERENCES

Andervont, H. B. Further Studies on Production of Dibenzanthracene Tumors in Pure Strain and Stock Mice. Pub. Health Rep., **50**:1211-1217. 1935.

Brunschwig, A., Tschetter, D., and Bissell, A. D. Wound Healing and Neoplasia. An Experimental Investigation. Ann. Surg., **106**:1084-1088. 1937.

Burrows, H. Experiments in Carcinogenesis. The Effect of the Subcutaneous Injection of Aseptic Irritants in Rats and Mice. J. Path. & Bact., **43**:427-428. 1936.

Choldin, S. Künstliche Prädisposition zum Teerkrebs. Ztschr. f. Krebsforsch., **31**:545-556. 1930.

Cramer, W. On Experimental Carcinogenesis: The Local Resistance of the Skin to the Development of Malignancy. Brit. J. Exper. Path., **10**:335-346. 1929.

Daels, F. Contribution à l'étude du Cancer expérimental. Congrès du Cancer, Strasbourg, **2**:54-55. 1923.

Deelman, H.-T. Quelques remarques sur le cancer expérimental du goudron. Bull. Assoc. franç. p. l'étude du cancer, **12**: 24-30. 1923.

Derom, E. Influence de la température sur la production du cancer expérimental du goudron chez la souris. Bull. Assoc. franç. p. l'étude du cancer, **13**:422-462. 1924.

des Ligneris, M. J. A. Precancer and Carcinogenesis. Am. J. Cancer., **40**:1-46. 1940.

Dietrich, D. Die Regeneration der Mäusehaut bei Vorbehandlung mit cancerogenen Stoffen. Ztschr. f. Krebsforsch., **48**:187-203. 1939.

Dittmar, C. Über die Empfänglichkeit der Haut und des Bindegewebes bei verschiedenen Mäusestämmen gegen carcinogene Reize. Ztschr. f. Krebsforsch., **50**:20-26. 1940.

Ludford, R. J. Skin Regeneration and Cancer. Brit. J. Exper. Path., **10**:193-196. 1929.

Orr, J. W. The Effect of Interference with the Vascular Supply on the Induction of Dibenzanthracene Tumours. Brit. J. Exper. Path., **16**:121-126. 1935.

Parodi, U. Sulla produzione sperimentale del carcinoma da catrame. Pathologica, **16**:175-187. 1924.

Raposo, L. S. Les deux facteurs de la genèse des carcinomes. Compt. rend. Soc. de biol., **98**:1001-1002. 1928.

Reding, R. Influence de quelques métaux sur le développement du cancer du goudron. Compt. rend. Soc. de biol., **89**: 817-818. 1923.

Rondoni, P., and Corbellini, A. L'azione cancerogena delle dibenzoacridine e l'ustione come fattore realizzante. Tumori, **22**:106-118. 1936.

Roussy, G., Leroux, R., and Peyre, Ed. Influences des facteurs d'irritation locale dans le cancer du goudron chez la souris. II. Bull. Assoc. franç. p. l'étude du cancer, **13**:587-589. 1924.

Teutschlaender. Über Technik und Ergebnisse der experimentellen Krebserzeugung. Strahlentherapie, **15**:812-821. 1923.

Truffi, M. Produzioni di tumori da catrame nel topo in zone successivamente trattate. Riforma med., **40**:985-988. 1924.

Reactions of Hybrid and Parabiontic Pseudo-Hybrid Mice to Inoculations of Tumor C198*†

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INTRODUCTION

The following paragraphs report further studies of a transferable hepatic neoplasm (C198) which was first described in 1939 (1). This tumor, which originated spontaneously in an old breeding female of the leaden strain of mice, was first seen in 1936 and has been under observation up to the present time. As previously reported, C198 grows readily in mice of the C57 leaden strain in which it originated but not in mice of other inbred strains.

Certain additional experiments, which followed this work and which will be discussed later, frequently altered the natural response of inbred mice of the C57 black strain to injections of tumor C198. In this way normally resistant mice were made susceptible to the growth of the tumor. As this changed response bore a resemblance to the susceptibility seen in F_1 hybrids between the leaden strain and mice of the other strains, we have employed the term "pseudo-hybrid" to describe such an animal showing an altered response. Previously we used "pseudo-hybrid" in describing the increased susceptibility of descendants of transferred-ova mice [1] to implants of tumors originating in the same strain that was employed as the ova-recipient foster mother strain (2).

A pseudo-hybrid could be defined as an inbred animal whose prenatal or postnatal environment has so altered its physiological response (or responses) that a change has occurred to give it some of the characteristics of a hybrid. This change is measurable, at least in part, through the use of transplantable spontaneous tumors to determine the susceptibility of the inbred strain, the pseudo-hybrid, and the true hybrid groups.

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† Read at the meeting of the American Association for Cancer Research, Boston, Massachusetts, March 31, 1942.

[1] Fertilized ova were removed from the ova-donor females of one strain and implanted into the uteri of ova-recipient females of a different strain. The mice mentioned here were descendants of mice obtained in this transfer.

MATERIALS AND METHODS

Tumor

Tumor C198 remains essentially the same clinically as it was 65 tumor generations ago when it was first seen as a spontaneous reticuloendothelioma. The original leaden strain host was sacrificed because of ascites and cachexia. At autopsy, the abdomen was filled with a serosanguinous fluid and the liver and spleen exhibited a pronounced tumorous condition. The spleen contained a few well defined, white, elevated tumor nodules. The whole liver was swollen, friable, grayish, and mottled with hemorrhagic foci, while the surface was roughened. No other organs appeared involved upon gross examination.

The histopathology which led to the diagnosis should be mentioned briefly. The liver showed a striking distension and partial to complete obstruction of many of the blood vessels, caused by irregular, compact, partially pigmented masses of cells in their lumina. The parenchymal cells, in general, retained their typical architecture but the sinusoidal spaces showed widespread small round cell infiltration. Megakaryocytes were rather frequent here and in livers from later tumor generations. The same condition existed in other types of tumors examined, such as carcinoma of the liver and lymphosarcoma. The tumor cells extended into the hepatic sinusoids but were most conspicuous in the larger blood vessels, where the cell masses were closely adherent to the endothelium. In longitudinal sections of the blood vessels these appeared as elongated thrombi. The tumor cells were quite uniformly large, some elongated and some polyhedral in shape, and frequently gave the impression of growing in radiating cords. These cells had an eosinophilic cytoplasm with a purplish tint, and phagocytized pigment and erythrocytes were frequently observed. The nuclei were hypochromatic, excentric, and usually oval or bean-shaped in outline. Mitotic figures were seen with fair frequency.

In the spleen tumor cells of the same type were seen growing within the blood vessels as irregular nests. They were also observed growing outside the vessels. Sometimes they were elongated into blunt

spindle cells arranged in a wavy swirling pattern. On the whole these cells resembled reticuloendothelial cells more than they did monocytes.

Method of Transfer

Inoculation was routinely carried out by implanting small pieces of involved liver with a trocar in the right axillary region of the recipient. The routine stock tissue was carried along in leaden mice. One of the characteristics of this tumor was that in the early transplant generations there was usually no subcutaneous reaction, but from the 8th generation on subcutaneous masses developed at the site of injection in all leaden mice when tumor tissue was implanted under the skin. Regardless of the degree of subcutaneous reaction tumors regularly developed in the livers of the injected leaden mice.

Experimental Treatment of Animals

Parabiosis.—Parabiosis was employed first to study the reactions of C57 leaden with leaden (L-L) parabionts. Later this test was extended to C57 black with leaden (B-L) and also black with black (B-B) in parabiosis. The method involved joining together two animals in such a way that when the incisions healed their bodies had grown together and they lived side by side. The mice were anesthetized, their lateral body skins opened along adjacent sides, the pectoral and pelvic girdles sutured together firmly, but not too closely, and finally the skins of the two mice were sutured carefully all the way along both margins of the incisions. In this way the mice were united firmly and held until healing occurred. In this operation the body wall was neither cut nor sutured.

Other workers have used parabiosis in studies of leukemia (3).

This close union of animals in parabiosis is one possible method of environmentally altering their physiological reactions. Probably this is especially true when representatives of different stocks of mice are brought together. The following paragraphs describe an attempt to utilize a transplanted tumor as a means of detecting the possible presence of pseudo-hybrid physiological reactions through a study of susceptibility to tumor transplants in mice living in parabiosis.

Records.—Throughout these experiments individual records have been kept on all the mice. Weekly gross observations were made and in the late stages of the tumor daily observations were necessary. This has made available complete autopsy data on all individual mice in the last 20 tumor transplant generations. The records include the gross appearance of the lungs, liver, kidneys, gonads, spleen, and lymph nodes, as well as the tumor reactions at the site of implantation. Earlier records often include the same data but routine autopsy observations were not always recorded outside of the presence or absence of the tumor in the liver.

Animals.—The strains employed in this report were the C57 leaden, the C57 black, and first generation hybrids between them.[2]

Experimental Results Following Inoculation with Leaden Strain Tumor C198

The C57 leaden mice are uniformly susceptible, for the tumor originated in this strain. The first 10 tumor transplant generations are shown in Table I. In the first 3, 79 leaden mice ranging in age from 1 to 11 months were inoculated. All developed internal tumors in the liver. Only 6 of these 79 mice showed subcutaneous reactions at the site of injection; these subcutaneous masses were tiny and soft. In the 4th transplant generation the tumor began to grow more slowly. Some positive mice lived 10 months, but all eventually died of C198. Only in the 5th and 6th tumor generations did we find negative mice. Nine negative mice were killed and autopsied after they were in the spontaneous tumor age group. In the following tumor generations the neoplasm grew more rapidly and appeared like the original growth except that it always developed subcutaneous masses from the 8th generation on.

Table I: Reactions of Leaden (L) Mice to Subcutaneous Injections of C198

Tumor Generations 1 to 10

Tumor generation	(L) mice inoculated	Age at inoculation, months	Life span of + mice, days	Reactions: Subcutaneous +	Reactions: Subcutaneous −	Reactions: Internal +	Reactions: Internal −
1	10	1	40- 89	0	10	10	0
2	14	1- 3	36- 52	2	12	14	0
3	55	1-11	30- 95	4	51	55	0
4	32	1- 5	48-309	4	28	32	0
5	17	1- 2	83-447	0	17	12	5 *
6	27	2- 5	61-301	6	21	23	4 **
7	12	1	61-187	1	11	12	0
8	6	1	58- 73	6	0	6	0
9	12	1	44- 71	12	0	12	0
10	12	1	33- 60	12	0	12	0
1-10	197	1-11	30-447	47	150	188	9

* Negative mice killed (1 at 492 days and 4 at 595 days).
** Negative mice killed (all 4 at 444 days).

[2] As yet no significant difference in reaction to tumor C198 has been recorded between reciprocal hybrids, so that in this paper they are all grouped together regardless of the direction in which the cross is made.

Table II presents the 11th to the 65th tumor generations in leaden mice. Here over a thousand mice showed 100 per cent susceptibility. The life duration after inoculation was 3 to 4 weeks. Except for 9 mice in the 5th and 6th tumor generations, tumor C198 always developed in the liver of leaden mice following subcutaneous injections of tumor tissue.

TABLE II: REACTIONS OF (L) MICE TO SUBCUTANEOUS INJECTIONS OF C198

TUMOR GENERATIONS 11 TO 65

Tumor generation	(L) mice inoculated	Average life span, days	Reactions: Subcutaneous +	Subcutaneous —	Internal +	Internal —
11-15	105	46	105	0	105	0
16-25	178	31	178	0	178	0
26-35	229	28	229	0	229	0
36-45	271	24	271	0	271	0
46-55	212	26	212	0	212	0
56-65	184	25	184	0	184	0
11-65	1,179	30.2	1,179	0	1,179	0

TABLE III: REACTIONS OF BLACK (B) MICE TO SUBCUTANEOUS INJECTIONS OF C198

Tumor generation	(B) mice inoculated	Life span after inoculation, months	Reactions: Subcutaneous +	Subcutaneous —	Internal +	Internal —
3-4	45	3.0-19.0	0	45	0	45
6	18	6.5-13.5	0	18	0	18
22-52	55	6.5- 7.5	0	55	0	55
3-52	118	3 -19	0	118	0	118

The C57 black strain is the other inbred strain that was inoculated with the tumor, and Table III shows the result of injecting C198. Only 3 mice were killed at 3 months; the rest lived 6.5 to 19 months. All were autopsied at death and all were negative. Thus the black strain is entirely resistant to the leaden tumor C198.

The F_1 hybrids between susceptible leaden and non-susceptible black strains were also tested. Table IV gives the result of injecting C198 into 104 of these hybrids. As we observed also in the leaden strain, the only negative mice were in the 5th and 6th tumor generations. In all subsequent tumor generations 100 per cent of the mice had subcutaneous and internal tumors. These mice lived longer and, as is shown later, developed the tumor in their kidneys and ovaries.

The susceptibility of leadens, blacks, and their F_1 hybrids is summarized in Table V. Leaden and hybrid data show the uniformity with which the tumor grows in susceptible mice. The gross appearance of these three groups of mice following injections of C198 is seen in Fig. 1. In the leaden mice at 3 to 4 weeks after subcutaneous implantation of the tumor the liver was always swollen and hemorrhagic; it might or might not be nodular. The lungs were usually hemorrhagic, the spleen often contained definite white nodules, and the subcutaneous mass was relatively small. In the black mice there was no reaction, and in Fig. 1 a mouse of this strain is shown to be negative. Although the hybrid lived longer than the leaden, it usually failed to show liver, lung, or spleen involvement. On the other hand, the hybrid males and females showed extensive kidney nodules and the females nearly always had enormous tumorous ovaries. Subcutaneous masses were large in the hybrids.

TABLE IV: REACTIONS OF F_1(L × B) HYBRIDS TO SUBCUTANEOUS INJECTIONS OF C198

Tumor generation	F_1(L×B) mice inoculated	Life span of + mice, days	Reactions: Subcutaneous +	Subcutaneous —	Internal +	Internal —
5-6	19	58-323	0	19	9	10 *
14	20	83-116	20	0	20	0
19-71	31	21- 71	31	0	31	0
66	34	20- 48	34	0	34	0
5-66	104	20-323	85	19	94	10

* Note similarity to same tumor transplant generations in pure (L).

TABLE V: SUMMARY TO REACTIONS OF (L), (B), AND F_1(L × B) HYBRIDS TO SUBCUTANEOUS INJECTIONS OF C198

Type of mice	Tumor generation	Number of mice inoculated	Average life span after inoculation, days	Reactions: Subcutaneous +	Subcutaneous —	Internal +	Internal —
(L)	11-65	1,179	30.2	1,179	0	1,179	0
(B)	3-52	118	229.8	0	118	0	118
F_1(L × B)	14-66	85	59.8	85	0	85	0

When susceptible leaden mice were in parabiosis (L)-(L) and one member only was inoculated with C198, both members developed the tumor in their livers (Table VI). This was true of all 23 pairs

employed. However, the uninoculated member showed early changes when the host was quite advanced. Length of life was the same as for (L) not in parabiosis.

showed no response. When the C57 black member of the (B)-(L) parabiont combination was inoculated with C198 both the (B) and the (L) members usually developed the tumor (Table VII). Of 47 pairs only

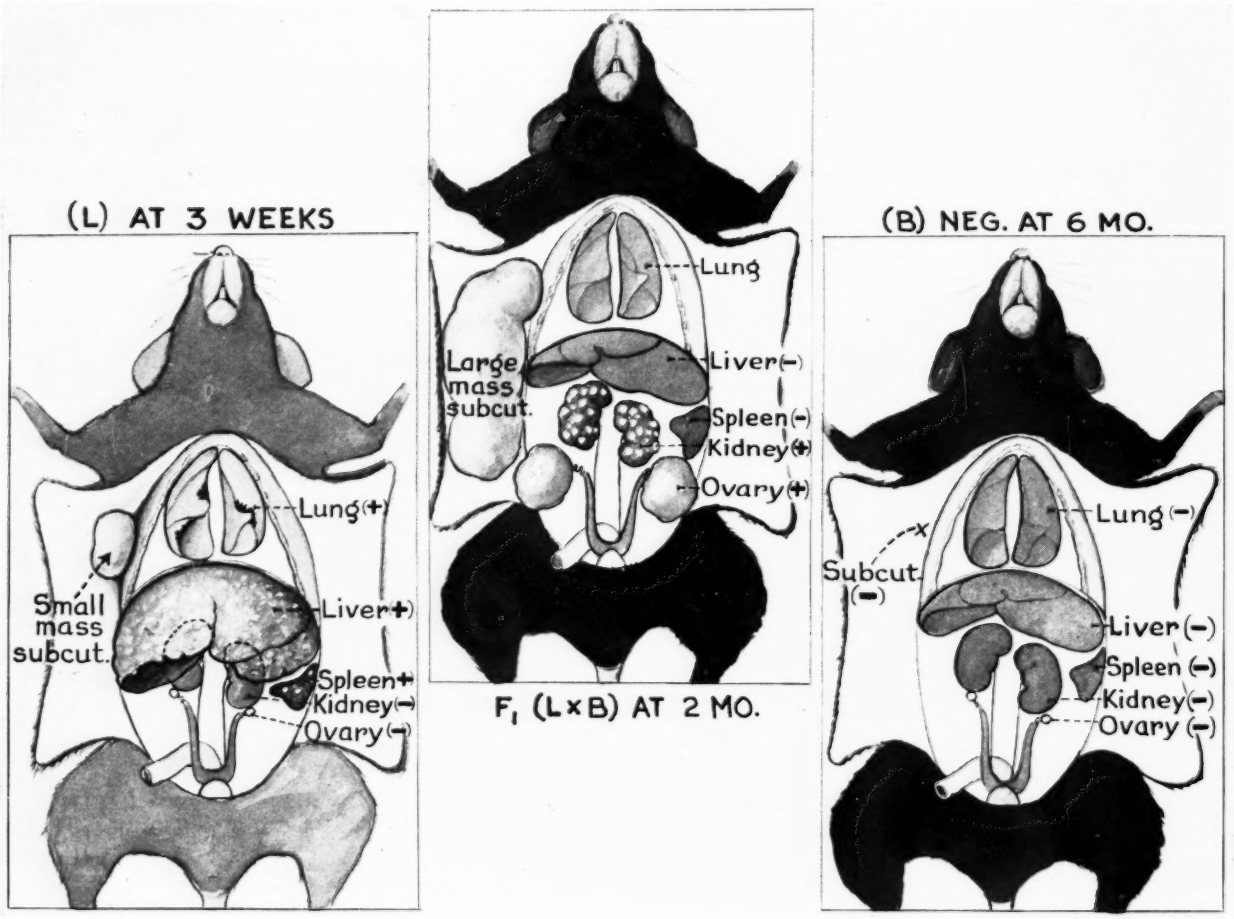

FIG. 1.—Reactions of leaden (L), black (B), and their $F_1(L \times B)$ hybrid to subcutaneous inoculations with (L) tumor (C198).

TABLE VI: PARABIOSIS OF LEADEN WITH LEADEN, 23 PAIR (L)-(L). RIGHT MEMBER ONLY HAD SUBCUTANEOUS INJECTION OF C198. REACTIONS OF BOTH MEMBERS AFTER INOCULATION INTO ONE

Stock and sex	Life span after inoculation, days	Reactions in (L)-(L): Right (L) inoculation: Subcutaneous +	Subcutaneous −	Internal +	Internal −	Left (L), internal +	Left (L), internal −	Reactions (L)-(L): Right (L) +	Reactions (L)-(L): Left (L) +
12(L♂)-(L♂)	21-27	12	0	12	0	12	0	12	12
11(L♀)-(L♀)	20-30	11	0	11	0	11	0	11	11
23(L)-(L)	20-30	23	0	23	0	23	0	23	23

Nonsusceptible black mice in parabiosis with susceptible leadens (B)-(L) also were tested with C198. The mice were united and the incisions allowed to heal before the tumor was injected. When the susceptible leaden member was inoculated, the leaden reacted like a leaden not in parabiosis and the black

3 were shown to be truly negative in both members. When the mice that died 3 to 18 days after inoculation are omitted, the corrected total is 34 pairs on which we have data.

Thirty of the 34 black mice developed a subcutaneous mass at the site of injection, and about half

of these showed liver masses. When these mice lived 1 to 2 months the leaden members developed the tumor in their livers.

developed the tumor more extensively and died first. The tumor did not later regress in the (B) mouse even if the leaden member was surgically separated from it.

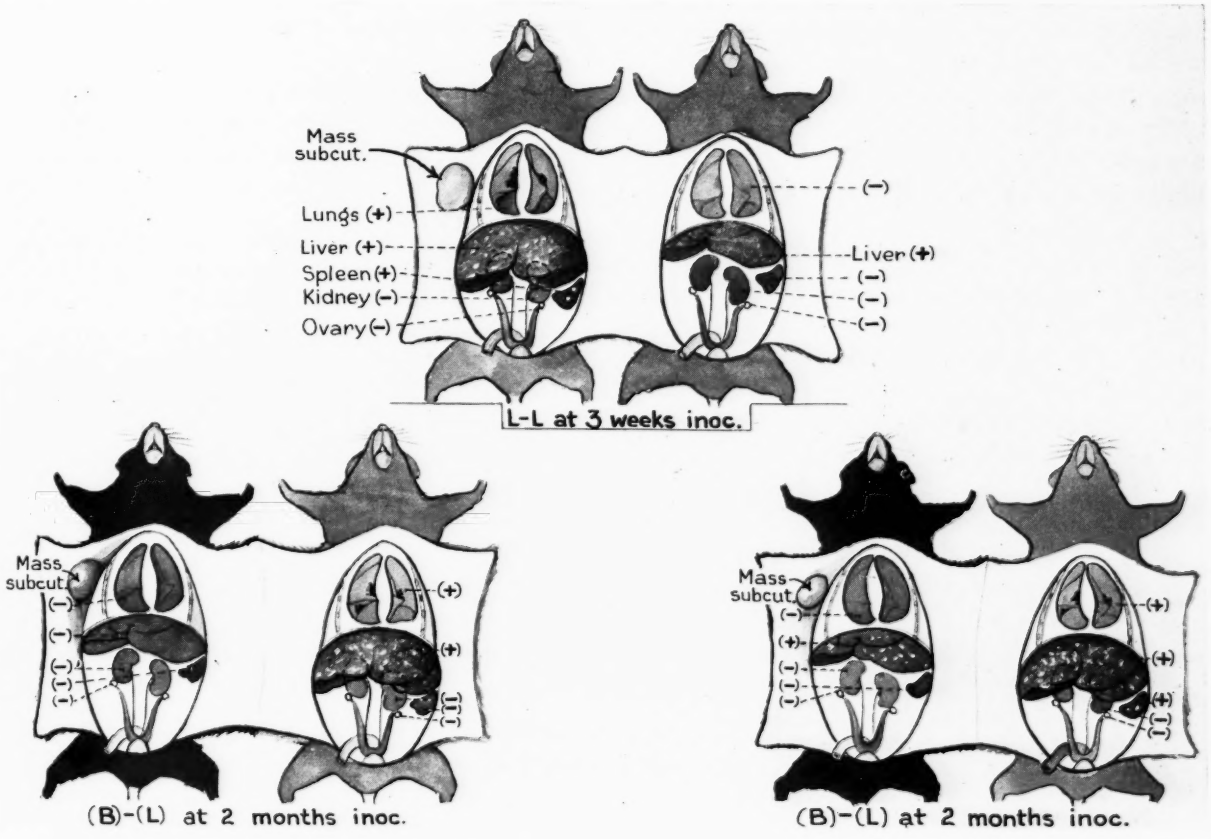

FIG. 2.—Parabiontic combinations of leaden (L) and black (B) mice (L)-(L) and (B)-(L). Reactions of both members to C198 following subcutaneous inoculation into only one member of a pair. Inoculation site shown by "Mass subcut."

TABLE VII: PARABIOSIS OF NONSUSCEPTIBLE (B) WITH SUSCEPTIBLE (L), (B)-(L). BLACK (B) MICE ONLY HAD SUBCUTANEOUS INJECTIONS OF C198. REACTIONS OF BOTH MEMBERS AFTER INOCULATION INTO ONE *

Stock and sex	Reactions to C198 in 47 (B)-(L) parabiont pairs									
	B s+i+	L i+	B s+i−	L i+	B s+i−	L i−	B s+i+	L i−	B s−i−	L i+	B s−i−	L i−
	pairs		pairs		pairs		pairs		pairs		pairs	
26(B♂)-(L♂)	5		6		4		1		1		9	
11(B♀)-(L♀)	5		4		0		0		0		2	
6(B♂)-(L♀)	2		2		0		0		0		2	
4(B♀)-(L♂)	1		0		0		0		0		3	
47(B)-(L)	13		12		4		1		1		16	
34(B)-(L) **	25(B+)-(L+)				5(B+)-(L−)				1(B−)-(L+)		3 † (B−)-(L−)	

* In the blacks the subcutaneous and the internal reactions are recorded but only the internal reaction of the leadens is given. s=subcutaneous and i=internal reactions (s+=subcut. tumor).

** In corrected total 34 pairs (B)-(L) all + mice died 30-58 days after inoculation.

† Out of 16 pairs (B−)-(L−) all but 3 pairs died too soon. Three known (B−)-(L−) died at 52, 70, and 75 days.

A comparison of these two types of parabionts, (L)-(L) and (B)-(L), is shown in Fig. 2. In the (L)-(L) combination the inoculated mouse died first. When the (B) member of the (B)-(L) parabiont combination was inoculated the leaden mouse (L)

A few (B)-(B) parabionts were inoculated with C198 as controls. In only one case an inoculated parabiont (B) mouse developed a small mass at 3 months, but this later disappeared. At 8 months all (B)-(B) mice were negative.

DISCUSSION

Different strains of mice can be united in parabiosis and they frequently survive for long periods; one pair lived for 13 months before they were accidentally killed. The data discussed above show that parabiosis between different strains can measurably change the physiology of the mice involved. In the detection of such a change a transplantable tumor is a useful gauge.

The susceptibility to C198 is not altered by the age of the host. Data not included show that leaden mice 1 to 15 days of age react similarly to those here described. Sex does not influence susceptibility, either in single mice or in parabionts. Reciprocal F_1 hybrids between leaden and black strains show no differences in susceptibility to C198.

Some influence has been transferred to the blacks, while in parabiosis with the leadens, that enables most of the black mice to develop C198 following their inoculation with that tumor. What this influence is cannot yet be determined. However, it can be detected and studied.

CONCLUSIONS

1. Parabiosis between different stocks of mice is possible.

2. Parabiosis measurably changes to susceptibility the normal resistance of black mice to transplants of tumor C198.

3. A transplantable tumor can be conveniently used as an indicator, constant in its own characteristics, to detect certain physiological changes in a mouse.

4. The changed reaction of a previously nonsusceptible mouse following parabiosis with a susceptible mouse indicates a response like that of an F_1 hybrid and the result is called pseudo-hybridization.

REFERENCES

1. Cloudman, A. M. A Transferable Liver Neoplasm (C198) Arising in a Female Mouse of the Leaden Strain. Am. J. Cancer, **36**:578-580. 1939.
2. Fekete, E., and Little, C. C. Observations on the Mammary Tumor Incidence of Mice Born from Transferred Ova. Cancer Research, **2**:525-530. 1942.
3. Furth, O. B., Barnes, W. A., and Brower, A. B. Studies on Resistance to Transmissible Leukemia in Mice by Means of Parabiosis. Arch. Path., **29**:163-174. 1940.

A Transplantable Squamous Cell Carcinoma in the Rabbit*

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Despite the frequency with which squamous cell tumors have been induced experimentally in the skin of the rabbit, spontaneous growths of this nature occur with great rarity. A review of the literature yields a single reported case (1). The tumor in this instance originated in the scar of a syphilitic chancre in the skin of the scrotum and, following transplantation and elimination of the syphilitic infection, became one of the most extensively studied neoplasms in cancer research.

The apparent low incidence of such growths in the rabbit is supported by the fact that observation of a large colony extending over a period of more than 10 years has disclosed only two instances. One of these, under the care of Dr. Louise Pearce, arose in the skin of the tail, but further study was terminated by death during the course of the first biopsy and transfer was unsuccessful. The other tumor, which originated in the skin of the cheek, was followed from inception to metastasis and transplantation was successfully effected. In view of the scarcity of such growths and because of several peculiarities of behavior in the spontaneous host and in experimental animals, an account of this tumor seemed desirable. Accordingly the pertinent data have been assembled and are reported in the present paper.

MATERIALS AND METHODS

The animal bearing the tumor was a female of Polish type derived from a line representing a concentration of Polish factors after a previous cross between the Polish and Himalayan breeds. Her immediate family history is of particular interest in the present connection inasmuch as her mother died of a generalized lymphosarcoma while, on the paternal side, the grandmother's death was due to metastases from an adenocarcinoma of the uterus and the grandfather bore a large bile duct adenoma. No relationship existed between this rabbit and the animal bearing the previously mentioned squamous cell tumor of the skin of the tail.

The animal was not subjected to special treatment but shared the same general conditions under which the remainder of the colony was maintained. She was first mated at the age of 5½ months and bore 3 litters totalling 9 young before discovery of the tumor 12 months later. Frequent observations were made after discovery of the growth and in several instances these were supplemented by biopsy and attempts to transfer tumor fragments to normal rabbits. A complete autopsy was performed following death, and tissues were procured for histological study.

The anterior chamber of the eye and the testicle were utilized as inoculation sites. The technic of anterior chamber transfer has been described (2) and the methods employed in testicular inoculation were similar to those in general use.

The Spontaneous Tumor

Clinical course.—The tumor was noted first on Oct. 21, 1940, when the animal was 18 months old, as a small scaly area in the skin near the base of the right ear. The general appearance was identical with that of a fungus lesion prevalent in the colony and, at the time, nothing was observed to suggest a different nature. The animal was mated and during the subsequent gestation, which terminated with the birth of 2 young on Nov. 22, the lesion increased in size and was transformed into an indurated translucent plaque of a coppery pink color covered with fine scales. The rapid growth continued, and on Dec. 3 it presented as an oval mass measuring 1.5 × 1 cm. projecting approximately 0.5 cm. above the level of the surrounding skin. The surface was covered with a thin brown scab which had been scratched away in areas, exposing a raw granulomatous tissue. The skin immediately adjacent was pale and covered with white epithelial scales while, at the extreme outer margin, a collar of redness surrounded the mass. The appearance of the lesion, as noted at the time, was strikingly like that of a syphilitic chancre. A biopsy was per-

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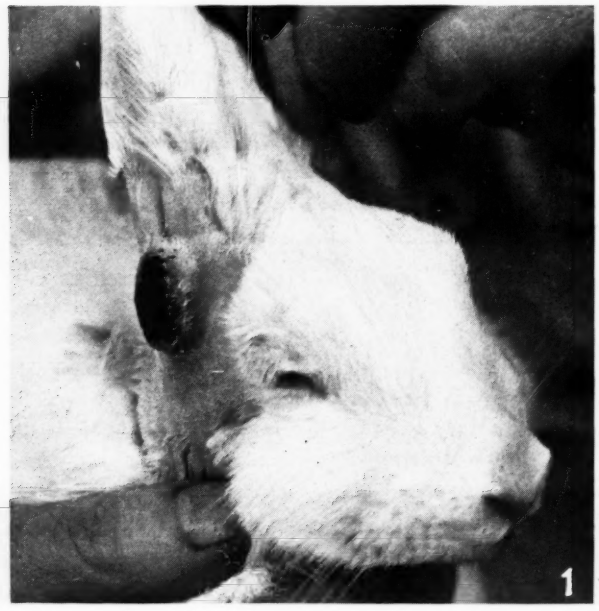
† Dr. Brown died August 4, 1942.

formed, however, and histological examination established its neoplastic nature.

Growth was greatly accelerated following biopsy and a week later the mass had increased by 0.5 cm. in all diameters (Fig. 1). There were no enlarged lymph nodes or other evidences of metastasis at this time and the animal appeared in excellent condition. A second biopsy was performed and fragments of tissue were removed for histological study and for transfer.



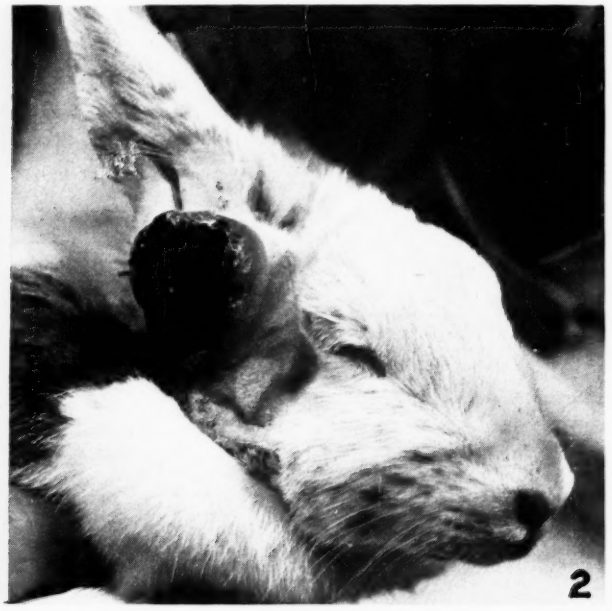


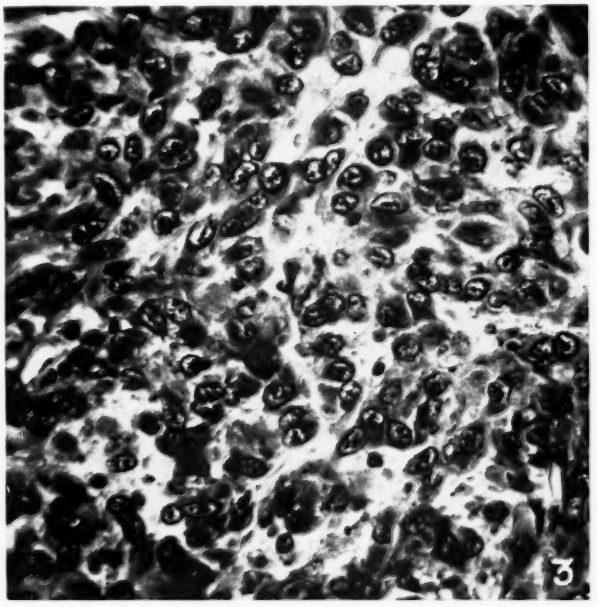
FIG. 1.—The primary tumor on Dec. 7, 1940, approximately 7 weeks after discovery. Mag. × 0.63.
FIG. 2.—The primary tumor on Jan. 9, 1941, one week before death. Mag. × 0.63.

On Dec. 20, an enlarged node was found at the angle of the jaw, the mass had increased another 0.5 cm. in diameter, and the animal had lost 125 gm. in weight. However, more progeny were desired to carry out genetic studies and a mating was made. Pregnancy followed but failed to check the growth rate and on Jan. 3, when a third biopsy was performed, the mass measured 3 × 2.5 × 2 cm.

The animal remained in fair physical condition despite continued weight loss until Jan. 9 (Fig. 2), when rapid deterioration began. This continued until Jan. 16 when, following the abortion of a litter of 6 young, she was killed by air injection.

Biopsies.—Biopsy specimens were obtained on Dec. 3 and 10, 1940, and on Jan. 3, 1941, by means of radially directed sections which included both peripheral and central portions of the tumor. Histologically, no significant differences were noted in the degree of cellular anaplasia or in architectural arrangement and the various specimens will therefore be described together.

The central portions of the tissue fragments were devoid of surface epithelium and covered by an amorphous layer of necrotic debris. Directly beneath this, normal structures were replaced by a growth of squamous cells which extended downward and laterally to the limits of the section. The predominant cells were round in shape with abundant cytoplasm and oval vesicular nuclei containing prominent nucleoli (Fig 3). Such cells were arranged with no indication of structural pattern, but here and there they were



All sections were stained with hematoxylin and eosin.
FIG. 3.—Section of the primary tumor obtained at biopsy on Dec. 3, 1940, showing squamous cell carcinoma. Mag. × 325.

partially separated into indistinct lobular masses by interlacing bands of spindle-shaped cells with regularly placed parallel nuclei. Mitotic figures were numerous in both cell types, and there was no indication of pearl formation or keratinization.

Toward the periphery of the tumor the surface epithelium was intact but the cells were generally thin and atrophic. The connective tissue of the dermis was greatly thickened and contained scattered nests of squamous cells together with upward extensions of the underlying tumor. Frequently such extensions followed hair follicles and gave rise to a striking histological picture. At the lower extremity of the follicle, tumor cells lined the space between the follicular epithelium and the surrounding connective tissue for some distance without invasion of either. Then with invasion and rupture of the epithelium the tumor grew into the lumen, expanding the follicle to a bombous sac filled with a dense cellular plug. In other areas the tumor cells replaced follicular epithelium without rupture into the follicle, or permeated the perifollicular spaces to the surface epithelium, and in both regions invaded at scattered points to produce an exact replica of the condition referred to as intraepithelial carcinoma or carcinoma *in situ* (Fig. 4).

Autopsy.—At autopsy the primary tumor measured 4 × 3 × 3 cm. and was firmly adherent to the underlying muscle which, on dissection, was found to be extensively invaded. There was considerable necrosis in the center of the mass but the major portion of the tumor was firm and apparently living.

There were several small metastatic nodules in the skin about the third left nipple and numerous firm shotty areas were found throughout the lactating breast tissue.

Alterations in internal organs were limited to metastatic invasion and to an area of infarction in the left kidney. Gross metastases were present in the spleen and the lung contained innumerable scattered pea-sized nodules. Minute areas of lighter color and firmer texture were observed in the liver and kidney but their identity could not be determined from gross examination. The submental and deep cervical lymph nodes of the right side were partially replaced by tumor tissue, but other lymphatic involvement was not noted. Gross metastases were not found in other internal organs or tissues.

The adrenals and the thyroid were enlarged but the pituitary was small. The ovaries contained large corpora lutea and one dead fetus was found in the uterus.

Histologically, the primary tumor showed the same characteristics observed at biopsy with, however, a shift in the preponderant cell type from squamous to spindle shape. The transition was gradual in some areas and intermediary cell forms were found, but usually the change was abrupt and nests of squamous cells appeared as isolated islands surrounded by spindle cells in sarcomatous arrangement. In general the peripheral parts of the tumor, including the portion adjacent to the skin, were made up solely of squamous cells, while the central portion consisted only of spindle cells. An intermediate zone contained solitary squamous nests embedded in a dense network of spindle cells and transitional forms (Figs. 5, 6, and 7).

Many lymphatics and blood vessels at some distance from the main mass were plugged with tumor cells, all of which were of the squamous variety. In addition, there were small actively invading nests of such cells deep in the underlying muscle (Fig. 8).

All the metastases were of squamous cell type and there was no suggestion anywhere of the spindle cell change that characterized the primary growth, nor was there evidence of metastasis of cells of this type. Keratinization was absent and there was little necrosis or other degenerative change. Stroma was generally scanty and there was no indication of a peripheral connective tissue reaction.

Secondary growths in lymph nodes occurred as solitary cellular masses replacing a large part of the parenchyma by their bulk but showing little evidence of peripheral invasion. Stroma was more abundant than in other locations and contained areas of hyalinization. The remainder of the nodes showed little change and detached tumor cells were not found in lymphatic sinuses.

In contrast, metastatic growths in the lung and spleen were small and multiple and occurred diffusely throughout the organs. Moreover, the blood vessels of the lung and the sinuses of the spleen contained innumerable tumor thrombi and free tumor cells. Frequently the tumor cells in the spleen remained wholly inside the sinuses, with distortion of their walls but without extrasinusoidal extension. Here and there, however, active invasion was evidenced by the production of solid tumor nodules visible in the gross (Fig. 9). On the other hand, the presence of tumor thrombi in the lung was almost invariably associated with extravascular growth. The more extensive areas were found in close proximity to large blood vessels and bronchi, and consisted of irregular loose growths of tumor cells which tended to follow alveolar walls rather than to infiltrate their lumina. Lesions of this type were diffusely scattered throughout all lung fields and the intervening alveolar walls invariably contained focal microscopic areas of growth associated with small blood vessels.

The skin metastases observed upon gross examination were situated in the dermis and subcutaneous tissue and consisted of multiple closely packed masses

of squamous cells separated by fibrous septa. Other areas of skin involvement were not found.

Questionable lesions were observed in the lactating breast tissue during autopsy and microscopic examination confirmed their identity as metastases. Other microscopic metastases were found in the corpora lutea and in the endometrium, and in view of the physiological status of the animal this unusual distribution assumes significance.

Various forms of growth were observed in different breast sections (Figs. 10, 11, and 12). In some lobules the secretory epithelium of the acini was completely destroyed and replaced throughout its extent by invading squamous cells. This occurred without other disturbance in the architecture of the tissue and the resultant picture was again a replica of the condition in man sometimes referred to as carcinoma *in situ*. Elsewhere continued proliferation of the squamous lining cells filled the acinar lumen, producing a definite tumor nodule in which, however, the alveolar architecture of the tissue remained recognizable. In still other regions acinar walls were destroyed and the lobule was converted into a solid tumor similar to those found in the spleen.

Ovarian metastases were limited to the corpora lutea of the last pregnancy. They were not extensive and occurred only as small cellular masses grouped around the central cavity (Fig. 13). Endometrial metastases were also very small and were not numerous. They were situated directly beneath the lining epithelium and rarely involved the thickness of the mucosa (Fig. 14). Small fragments of tumor tissue were found free in the uterine veins.

True metastases, as evidenced by extravascular growth, were not found in the liver or kidneys, but sections of these organs contained multiple examples of the intravascular arrest and growth of tumor cells without external extension or invasion of the parenchyma. Many of the sinusoids of the liver contained solitary arrested squamous cells or were completely blocked by groups or chains of these cells in active proliferation. Adjacent liver cords were compressed and the sinusoids distorted but the tumor cells remained entirely within their boundaries and invasion was not present (Fig. 15). In like manner many of the glomerular capillaries of the kidney were plugged with tumor cells, and frequently the entire glomerulus appeared to be completely replaced. However, in no instance did the tumor extend beyond Bowman's capsule, and the parenchyma of the organ was not invaded (Figs. 16 and 17). In one area an arcuate vein was completely blocked by a vascularized mass of tumor, with infarction of the corresponding cortical zone (Fig. 18).

The adrenal cortex contained numerous scattered pale gray areas made up of vacuolated cells with pyknotic nuclei. Such areas were irregularly distributed but tended to be concentrated in the innermost cortical zone (Fig. 19). The thyroid acini were considerably enlarged and contained dense colloid. The pituitary showed no abnormality other than an increase in the thickness of the intermediate zone.

The Transplanted Tumor

Primary transplantation experiments.—The first attempt to transplant the tumor was made with material derived from the initial biopsy, performed on Dec. 3, 1940. Tissue from the interior was used and fragments were transfered to the anterior chambers of 8 normal rabbits. The animals were held under observation for 4 months but no growth occurred, and autopsy at the end of this period revealed either complete resorption of the fragments or death and hyalinization. A second transfer was attempted with similar material on Dec. 10 and again, despite long continued observation, no indication of growth was noted. However, a third attempt, made on Jan. 3, 1941, was successful and growth occurred in 100 per cent of the animals used. One of the animals was killed a month later, with its chamber one-quarter filled with tumor, and transfer to a second generation again gave rise to 100 per cent of takes.

At autopsy transfers were made to the anterior chamber, the testicle, and the skin, but eye transplants alone were successful. Tissues from various parts of the primary growth, a submental lymph node, and a lung nodule were used in the latter case, and a high percentage of takes resulted in all instances.

In view of the possibility of a virus etiology, emulsions of the primary tumor and various metastatic nodules were rubbed into the scarified skin of 15 normal rabbits. The animals were held under observation for 100 days and numerous sections were ob-

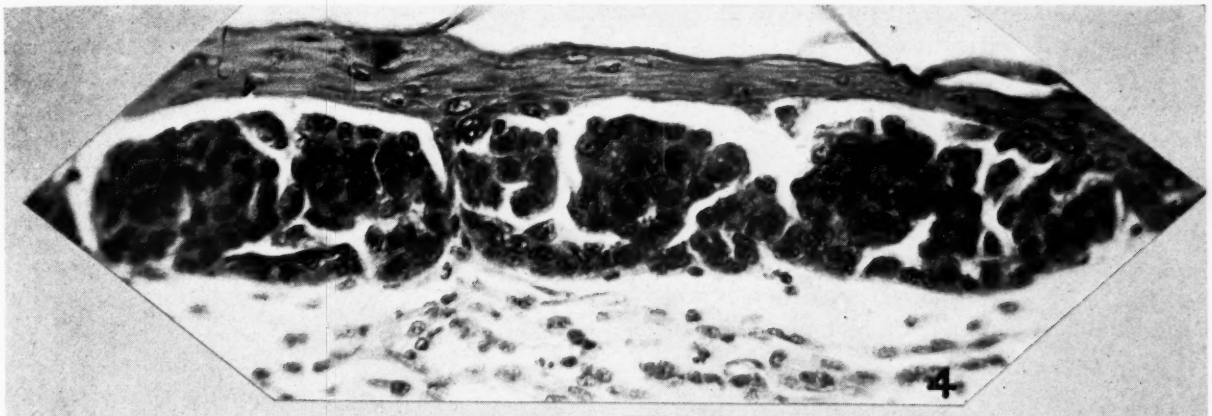
DESCRIPTION OF FIGURES 4 TO 8

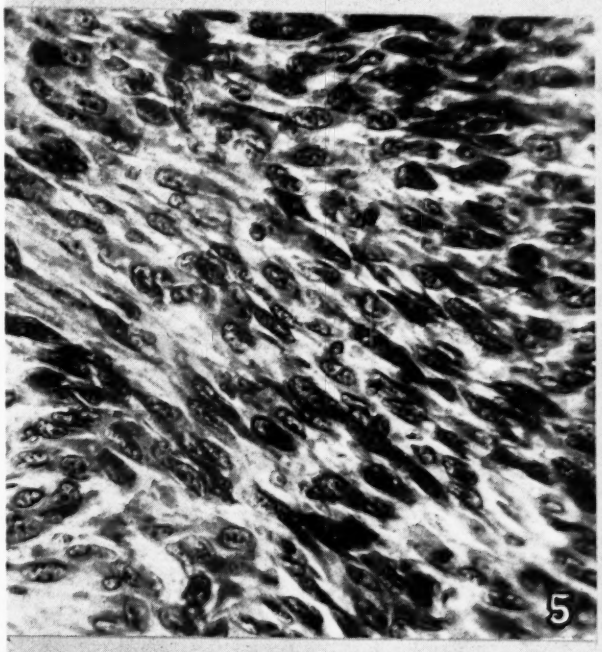
Fig. 4.—Section of skin obtained at autopsy. This area was separated from the primary growth by normal skin, and represents epithelial invasion rather than tumor origin. Mag. × 325.

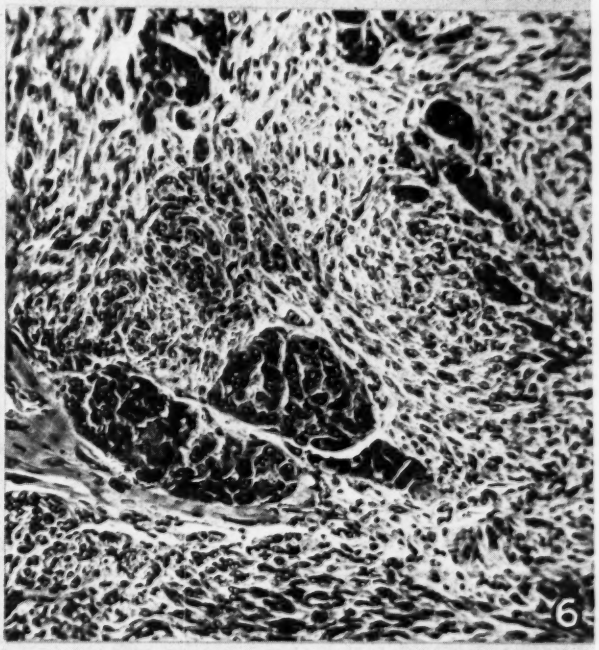
Fig. 5.—Section from central portion of primary tumor showing spindle cells simulating sarcoma in arrangement. Mag. × 325.

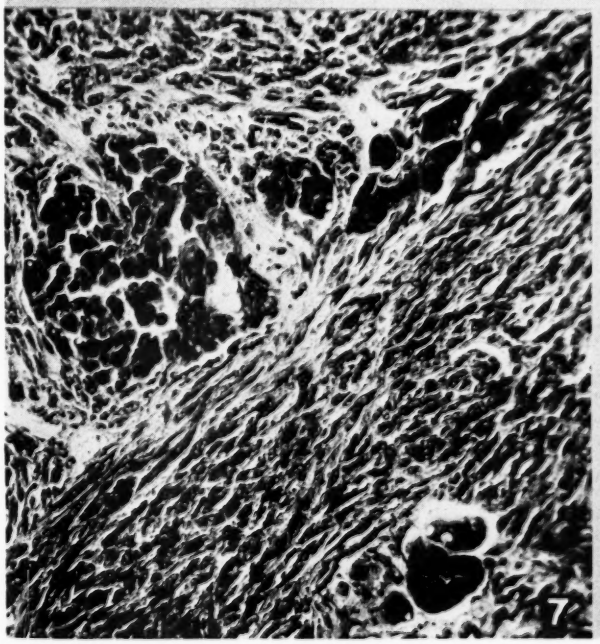
Figs. 6 and 7.—Sections of primary tumor showing islands of squamous cells surrounded by spindle cell growth. Mag. × 150.

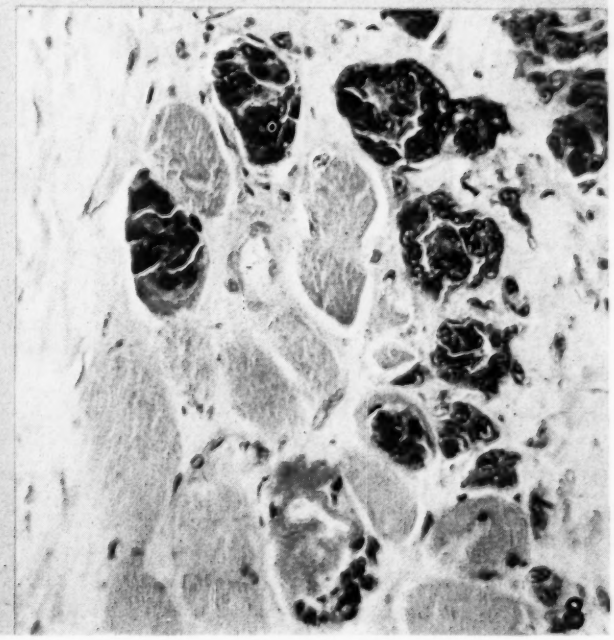
Fig. 8.—Section of muscle directly below the primary tumor showing active invasion and occupation of muscle bundles by squamous cells. Mag. × 260.











FIGS. 4-8

tained at autopsy. However, there was no indication on either clinical or pathological grounds of any change other than that incident to the trauma of scarification.

Serial transfer in the anterior chamber.—The results of primary transplantation experiments and of serial transfer in the anterior chamber of the eye are shown in Table I.

The secondary growth in the submental lymph node was selected for serial transfer, and as a rule this was performed as soon as the tumor filled the chambers of the generation under observation. It will be noted that until the beginning of April this required approximately 2 weeks and transfer was invariably successful in all animals. The 6th generation transfer, made on Apr. 9 as usual, gave rise to 100 per cent of takes, but growth was slow and the chambers were not filled until May 16. Moreover, retransfer on this date gave rise to only 37.5 per cent of takes. Growth occurred in all animals of the next 3 generations and at first was somewhat accelerated. However, a full month was required to fill the chambers of the 10th generation, and transfer to the 11th generation on Aug. 16 resulted in only 36.6 per cent of takes. Furthermore, the transplants in these animals grew very slowly. Two were killed when the tumor filled a quarter of the chamber but transfer was unsuccessful in both instances. The transplant in a third case underwent regression after 2 months of growth, and a fourth animal is still under observation. In this instance the fragment remained without perceptible change until May of 1942, and then began very slowly to increase in size. At the present time, 10 months after transfer, it occupies approximately one-fifth of the chamber.

TABLE I: THE RESULTS OF TRANSPLANTATION INTO THE ANTERIOR CHAMBER OF THE EYE

Date, 1940	Material used	Generation	Number of animals	Number of takes
Dec. 3	Primary tumor	1	8	0
Dec. 10	" "	1	6	0
1941				
Jan. 3	" "	1	6	6
Feb. 3	" "	2	5	5
Jan. 17	" "	1	9	9
Jan. 17	Lung metastasis	1	6	5
Jan. 17	Lymph node metastasis	1	7	7
Feb. 11	" " "	2	4	4
Feb. 24	" " "	3	5	5
Mar. 10	" " "	4	5	5
Mar. 24	" " "	5	10	10
Apr. 9	" " "	6	7	7
May 16	" " "	7	16	6
June 7	" " "	8	5	5
June 23	" " "	9	5	5
July 16	" " "	10	4	4
Aug. 16	" " "	11	11	4
Sept. 5	" " "	12	6	0
Sept. 14	" " "	12	6	0

In the majority of animals growth was apparent by the 8th day and progressed until the chamber was filled. Occasionally glaucoma developed during early stages of growth and was invariably associated with premature regression of the tumor. As a rule, however, regressive changes did not appear until the tumor filled the chamber, and in such cases living cells persisted for as long as 2½ months.

Testicular transfer.—Numerous attempts were made to transfer the tumor to the testicle and the results were similar in all cases. Takes occurred in the great majority of animals and the transplants grew rapidly to attain the size of a pea by the 10th day. Thereafter, however, regression invariably occurred and by the end of the 3rd week only residual scar tissue remained. Many attempts were made to transfer to a second generation at various periods after the 10th day but these were uniformly unsuccessful.

Morphology of Transplants.—At autopsy attempts were made to transplant both the typical squamous and the spindle cell types of tissue found in the primary growth. The cell type was determined by frozen section and the remainder of the block used for transfer was later sectioned serially. As a result of this procedure, it is certain that the transplanted fragments contained cells predominantly of one or the other type and in some instances were made up solely of one cell type.

No difference was noted in the growth behavior of the fragments in the anterior chamber and the progress and eventual fate of the transplants was the same in both cases. Moreover, on microscopic examination the resultant tumors were found morphologically identical, and irrespective of the original type of

DESCRIPTION OF FIGURES 9 TO 14

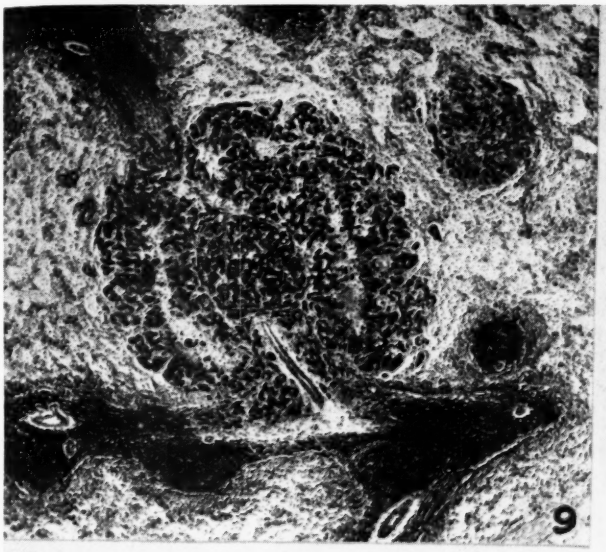
FIG. 9.—Section of spleen showing metastasis of squamous cells adjacent to a small blood vessel. Mag. × 150.

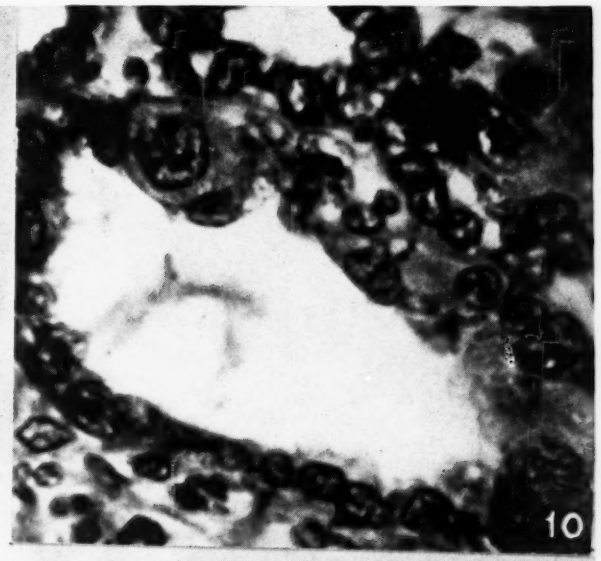
FIGS. 10 and 11.—Acini of breast showing replacement of lining columnar cells by invading squamous cells. Mag. × 925.

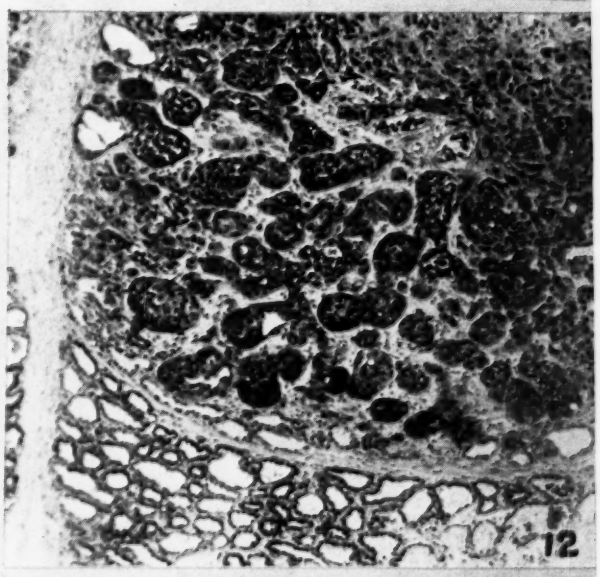
FIG. 12.—Section of metastasis in lactating breast showing extension of the process seen in Figs. 10 and 11. Some acini are completely lined by squamous cells while others are plugged by expanding growth. In one region, squamous cells have invaded the stroma, but in general they are limited to normal duct and acinar boundaries. Mag. × 75.

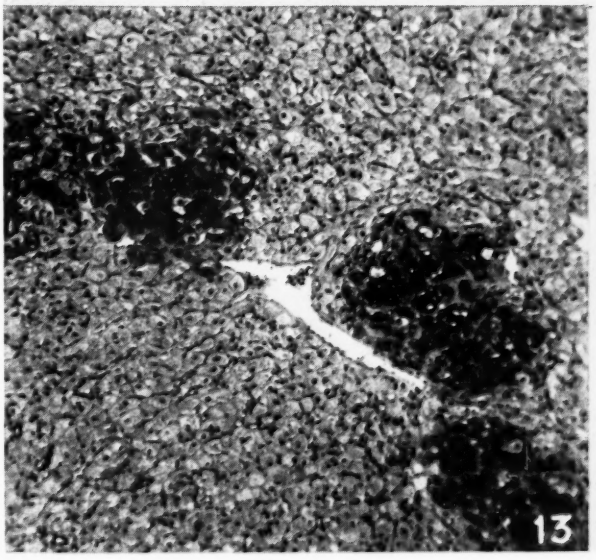
FIG. 13.—Section of metastasis in corpus luteum of pregnancy. Mag. × 75.

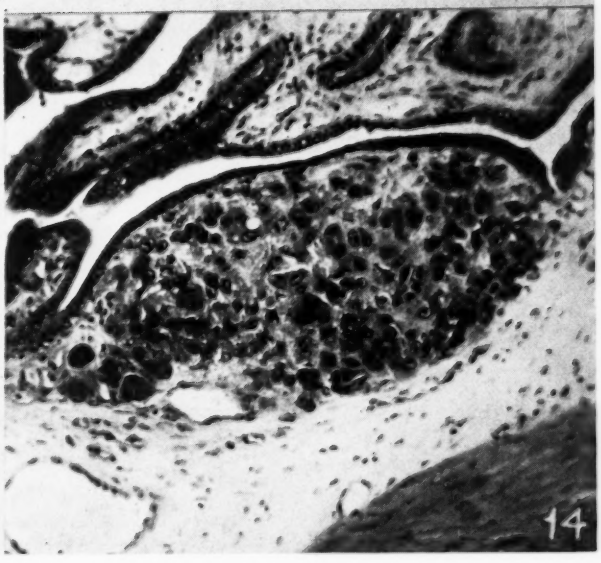
FIG. 14.—Section of metastasis in endometrium. Mag. × 150.











FIGS. 9-14

tissue used, whether squamous or spindle cell in character, the growths consisted entirely of squamous cells. There was no indication whatsoever of spindle morphology in the growths arising from transfer of fragments of that type, and the squamous character of the resulting tumor was in no way different from that of growths arising from the implantation of typical squamous fragments (Figs. 20 and 21).

The cells were large and flat, with abundant cytoplasm and oval vesicular nuclei containing prominent nucleoli. The nuclei varied widely in size and mitotic figures were numerous. There was no indication of keratinization. The cells were arranged in sheets without architectural pattern and the relationships were those of a squamous cell carcinoma. Blood vessels were numerous. Necrosis was present in scattered areas and formed a prominent feature of older transplants, where only those cells in the immediate vicinity of blood vessels remained living.

The iris and ciliary body were invaded and destroyed and occasionally deep infiltrations were found in the periretinal tissues, but extension into the posterior chamber was rare. Metastases or lymphatic extensions were never observed.

Animals with testicular growths were killed at various intervals after transfer in an attempt to determine the sequence of changes leading to early regression. It should be emphasized in this connection that testicular transfers were performed with cellular emulsions of anterior chamber growths, all of which were made up of squamous cells. However, the testicular tumors resulting from transfer of this material bore no morphological resemblance to the eye transplants and, with a single exception, were composed solely of spindle cells identical in structure and arrangement with those found in the primary spontaneous growth (Figs. 22 and 23). The exception occurred in the testicle of a pure bred Chinchilla, and here the tumor consisted of a mass of keratin surrounded by a thick fibrous capsule with a narrow inner lining of flattened cells (Fig. 24). The spindle cell growths invaded the testicular parenchyma and appeared healthy and capable of continued progression. However, in all instances degeneration and necrosis supervened and by the 20th day no evidence of growth remained and the tumor site was completely replaced by scar tissue.

DISCUSSION

Several points relative to the characteristics of the tumor in the spontaneous host and to its behavior in normal animals require further comment.

Morphologically the tumor corresponds to a relatively rare skin tumor in man known as spindle cell epidermoid carcinoma and characterized by a growth of spindle shaped cells in pseudosarcomatous arrangement (6). In man, the spindle character apparently may be present *ab initio* or may develop after a number of biopsies have shown typical squamous cell carcinoma. The histogenesis of the spindle cells is not clear, but morphological evidence leaves little doubt of their epithelial origin or of the carcinomatous nature of the tumor. The results obtained from transplantation experiments in the present instance offer biological confirmation of such observations.

The earliest biopsies of the tumor showed areas of spindle cell transition, but the preponderant cells were typically squamous in character. At autopsy, however, spindle cell growth dominated the picture, and it seems probable from morphological study that this growth proceeded from multiplication of pre-existing spindle cells as well as from an accelerated transition of squamous cells. It is of interest that the spindle cell change was limited to the immediate region of the primary growth and was not participated in by squamous cells in local or distant areas of extension or in metastases. It would seem probable, therefore, that the factors concerned in the change were strictly local in distribution. Moreover, the failure of the spindle cells to metastasize would indicate a lesser degree of autonomy than was attained by the squamous cells—a possibility which is not in harmony with the view that such cells represent a more anaplastic transformation.

Transplantation experiments confirmed the epithelial nature of the spindle cells and illustrated the reversible nature of the transformation. It was found that transfer of such cells to the anterior chambers of the eyes of normal rabbits gave rise to a squamous cell growth which was in no wise different from that produced by transplantation of typical squamous cells. Furthermore, transfer of the squamous cell growth from the anterior chamber to the testicle resulted in

DESCRIPTION OF FIGURES 15 TO 20

FIG. 15.—Section of liver showing squamous cells in sinusoids without parenchymal invasion. Mag. × 375.

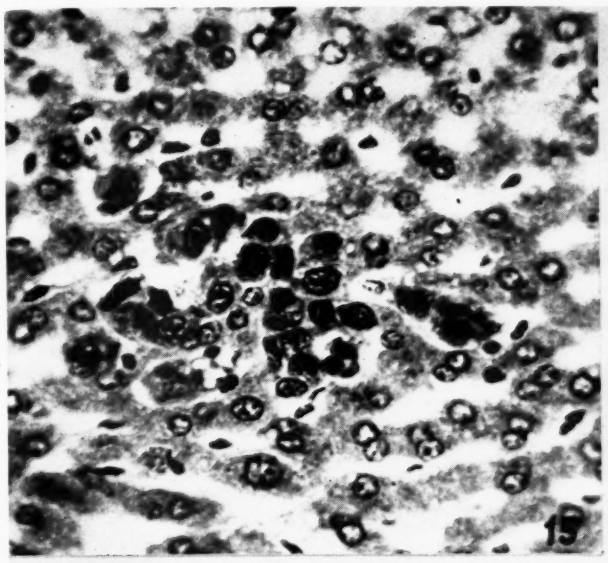
FIG. 16.—Section of kidney showing several glomeruli with capillaries completely occupied by intravascular proliferations of squamous cells. There is no extravascular growth. Mag. × 150.

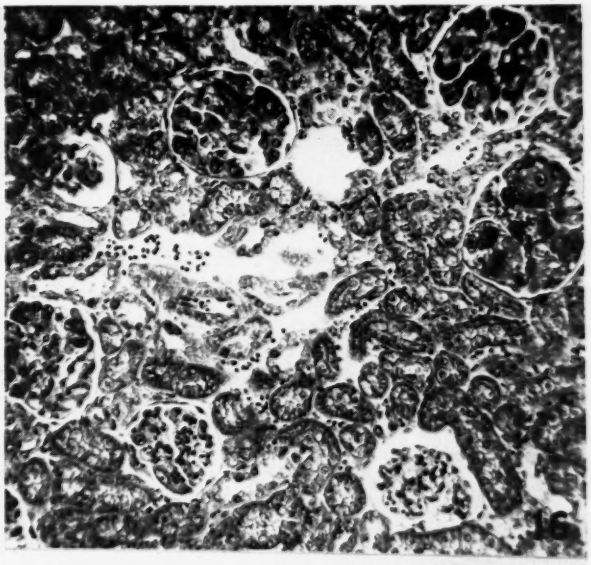
FIG. 17.—Higher power view of one glomerulus showing approximately one half the tuft occupied by squamous cells. Mag. × 300.

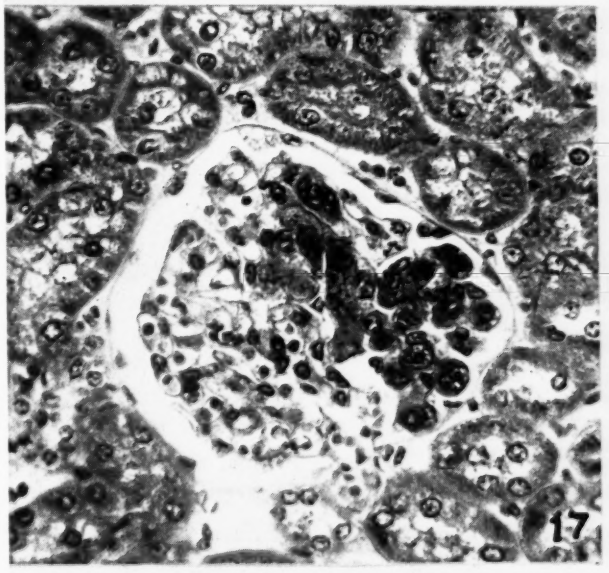
FIG. 18.—Section of kidney showing thrombus of squamous cells in arcuate vein with infarction of cortex. Mag. × 13.

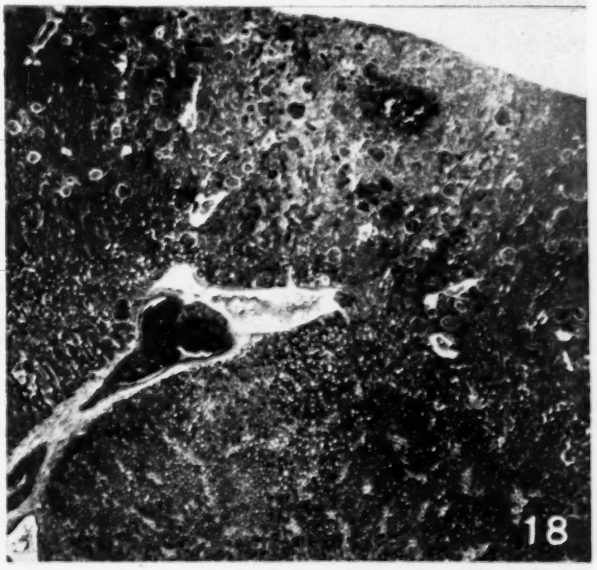
FIG. 19.—Section of adrenal cortex showing characteristic areas of degeneration. Mag. × 175.

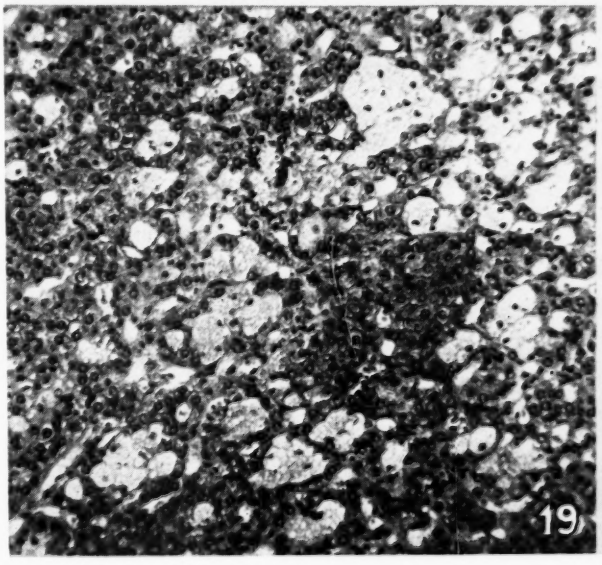
FIG. 20.—Section of transplant from anterior chamber of the eye showing growth of squamous cells. Mag. × 450.

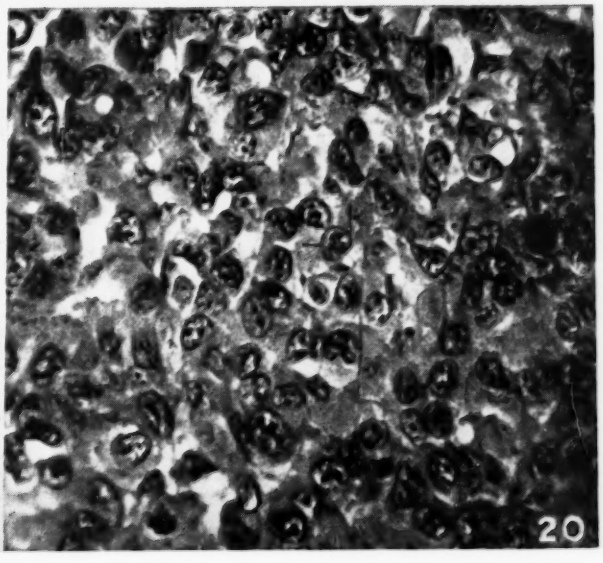












FIGS. 15-20

a reversion in cell type to the spindle shape and gave rise to a tissue identical with that found in the primary tumor. It is apparent, therefore, that the spindle cells were of epithelial nature and that the transformation was dependent on local factors. The natural extension of this observation is of interest inasmuch as it emphasizes the possibility that the degree of differentiation of a tumor may be influenced to a considerable extent by local environmental conditions. Its significance lies in the observed fate of growths made up of two cell types. Squamous cell growths in the anterior chamber progressed with active invasion and destruction until choked by the pressure incident to rapid expansion in a rigid space. On the other hand, spindle cell growths in the testicle underwent the developmental changes characteristic of normal squamous epithelium and terminated as dead masses of keratin.

The present study sheds no light on the nature of the factors concerned in the spindle cell transition or on the sequence of morphological changes leading to the transformation. It is of interest, however, that in unreported experiments (Greene) the transfer of human embryonic squamous epithelium to the guinea pig's eye frequently gives rise to spindle cell growths of similar character. It would appear, therefore, that potentialities in this direction are shared by embryonic as well as by neoplastic squamous epithelium. The ability of epithelium to simulate connective tissue in morphology is not a property peculiar to these cells. The reverse has also been observed—that is, the cells of a human fibrosarcoma after continued passage in guinea pig's eyes may assume a rounded shape and an adenoid arrangement so that a differentiation from epithelium on morphological grounds depends purely on subjective factors (3).

The primary tumor was also of interest in view of the extension of neoplastic squamous cells in the surface epithelium and in the epithelium lining hair follicles. This occurred at some distance from the primary focus of growth and obviously represented tumor extension rather than tumor origin or spread of the neoplastic process.

The occurrence of metastases in the lactating breast, the endometrium, and the corpora lutea appears highly unusual unless considered in relation to the fact that women or animals rarely become pregnant during the metastasizing phase of cancer. The distribution in the present case was undoubtedly determined by the increased vascularity and physiological activity of the parts incident to pregnancy.

The morphology of the breast lesions was particularly instructive. Here tumor cells replaced the normal columnar acinar lining exactly in the manner described in the case of neoplastic breast epithelium in instances of so called totilobular carcinoma. However, in the present example the squamous character of the neoplastic cells made it clear that the tumor extended by proliferation of neoplastic elements, with replacement and destruction of normal epithelium, rather than by a spread of the neoplastic process to adjacent cells.

This manner of extension was studied in a series of transplantation experiments and, in view of their present application, the results may be briefly mentioned. Normal rabbit breast tissue was transplanted to the anterior chambers of a number of animals and, after growth had become established, fragments of the tumor under discussion were placed in close proximity to the breast transplants. Actual invasion of the normal breast tissue did not occur but, on the contrary, the tumor cells extended along ducts and alveoli, replacing and destroying the normal epithelium exactly in the manner described above. On the other hand, if normal breast tissue from a rabbit bearing a spontaneous uterine cancer with metastases were used in place of tissue from a normal animal, invasion was widespread and a highly dissimilar histological picture resulted. It would appear, therefore, that the occurrence of epithelial extension in contrast to diffuse invasion was dependent upon the constitution of the tissue subjected to the tumor.

In the present instance it may be assumed that the breast tissue possessed a degree of resistance to invasion and that, primarily at least, tumor occupation occurred by way of epithelial extension. There was also evidence that other tissues of the spontaneous host possessed such resistance to an even greater degree. Definite invasive metastasis occurred in the lung and spleen, but in the liver and kidney tumor cells, while widespread in occurrence, were entirely intravascular in distribution and parenchymal invasion was completely absent.

Conceivably the resistance to invasion displayed in these organs bore relationship to the relatively short residence of the tumor in the animal. The spontaneous rabbit tumors studied in this laboratory rarely attain autonomy in less than 18 months, and at au-

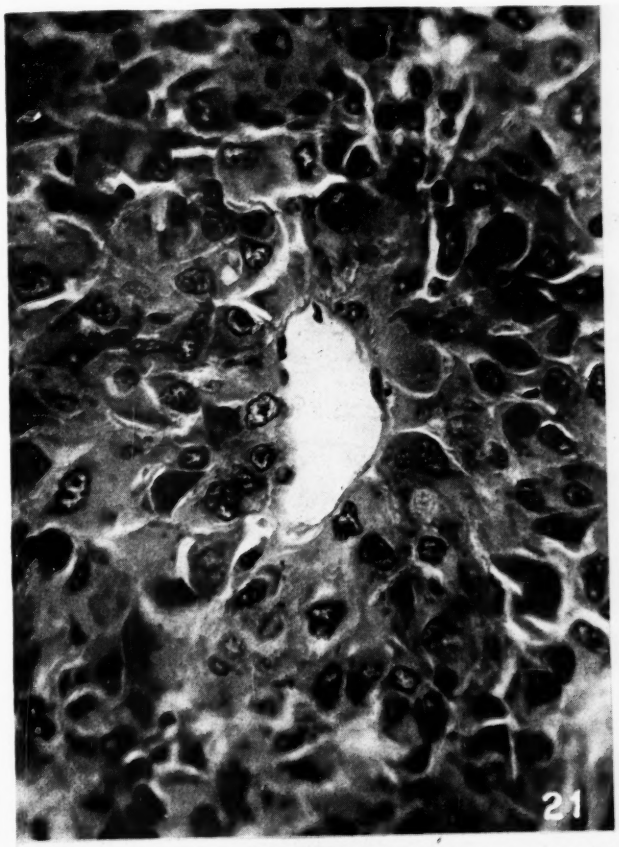
DESCRIPTION OF FIGURES 21 TO 24

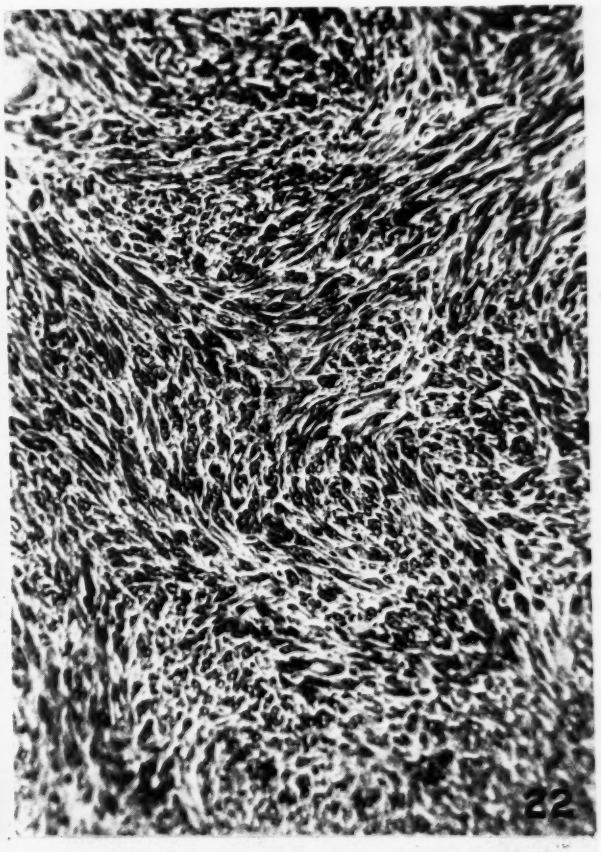
FIG. 21.—Section of transplant from the anterior chamber of the eye showing cells grouped around a thin walled blood vessel. Mag. × 325.

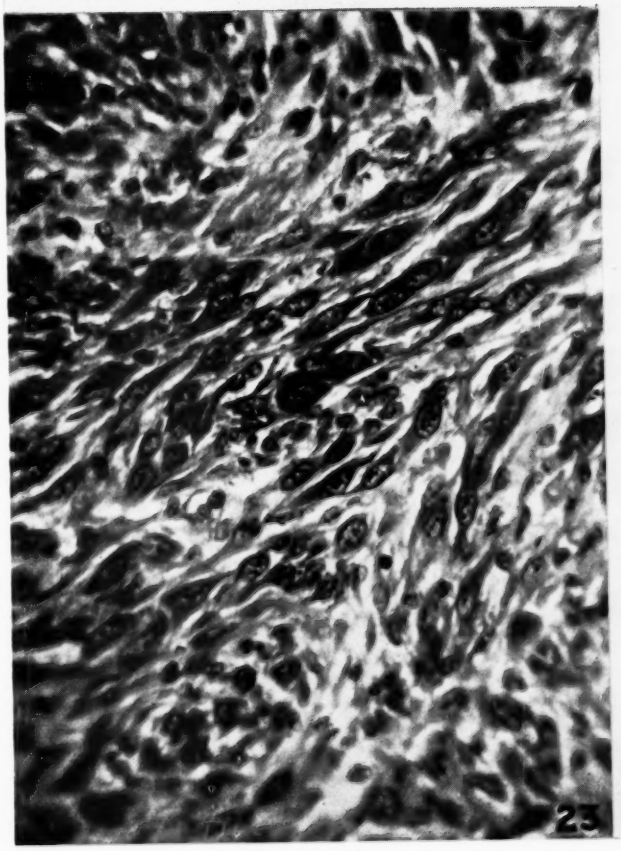
FIG. 22.—Section of testicular transplant showing growth of spindle cells in sarcomatous arrangement. Mag. × 150.

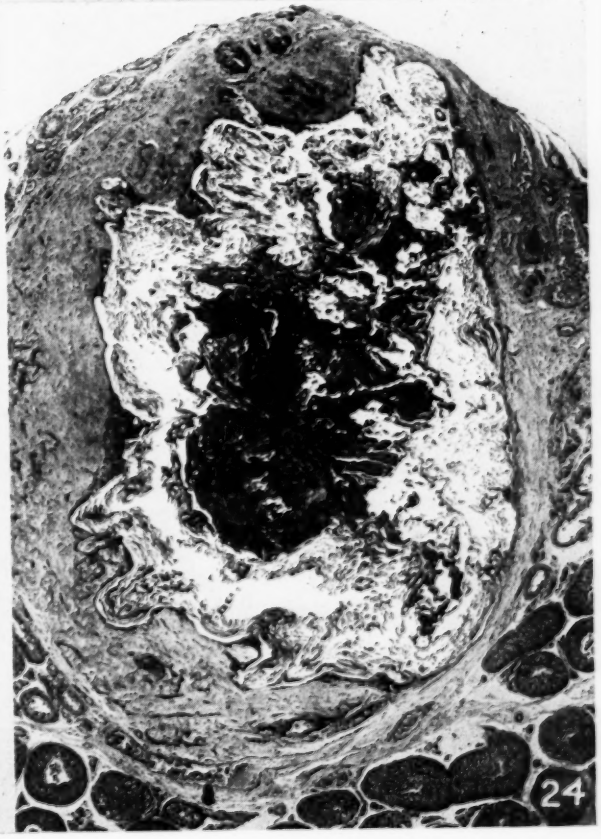
FIG. 23.—High power view of Fig. 22 showing more cellular detail. Mag. × 375.

FIG. 24.—Section of testicle showing fate of testicular transplants. The tumor cells have undergone keratinization and are surrounded by a thick fibrous capsule. Mag. × 35.









FIGS. 21-24

topsy true metastases are widespread throughout the body. In the present case autonomy, as determined by successful transfer, was attained in approximately 10 weeks, and the total residence of the tumor in the animal did not exceed 13 weeks. The situation in this respect is somewhat comparable to that which obtains following the transfer of many autonomous tumors to normal rabbits. Here long residence, up to a year in some instances, is essential before the occurrence of metastasis, despite progressive invasion and early rupture into blood vessels at the inoculation site.

The transplantation experiments undertaken at intervals after discovery of the growth gave results identical with those previously obtained with tumors of the breast and uterus (4). Early transfer to normal animals resulted in no growth and transplantation was only successfully effected after further development of the tumor. It would appear, therefore, that in tumors of the skin, as well as in tumors of the reproductive system, autonomy is only attained after continued development and is not associated with the primary neoplastic focus or with other evolutionary stages. In other words, and despite morphological appearance to the contrary, the primary tumor was not cancer in a biological sense at the time of the first biopsy and did not attain the biological characteristics that distinguish cancer until after continued development. Moreover the assumption of autonomous properties was not reflected in the morphology of the tumor cells.

A consideration of the possible factors concerned in the initiation of the neoplastic focus in the skin brings out several suggestive points. The inheritance of a genetic predisposition to cancer is indicated by the fate of the animal's forebears in both parental lines. The immediate exciting agent may have been chronic irritation incident to a fungus infection. However, the appearance of the adrenals was highly suggestive of an endocrine disorder comparable to that found in rabbits with mammary cancer (5). The changes were identical with those observed in rabbits subjected to long continued treatment with estrogenic substances, and it is conceivable that an increased or abnormal secretion of such substances occurred in the animal in question and played a part in the initiation of the neoplastic focus.

The greatly lengthened latent period that characterized the last transplanted generation of the tumor requires some comment. The interval between transfer and the appearance of signs of growth approximated 7 months in one instance, and in the ordinary course of events the animal might have been discarded as a non-take. This is not an unusual feature of anterior chamber transfers. The interval in the first generation transfer of human cancers to rabbits or guinea pigs usually exceeds 3 months, and homologous transplants often behave similarly. A transplantable Wilms' tumor of the rabbit under study regularly requires 6 to 7 months to manifest signs of growth and 12 to 18 months to fill the chamber. Again, transplants in animals released from experiments as non-takes and shifted to the breeding population have been observed, as long as a year later, to manifest signs of activity and eventually to grow and fill the chamber. The factors concerned in this variation are unknown but the phenomenon is of interest and importance and may be related to the well recognized calamity of recurrence of a human tumor many years after apparent complete extirpation.

Finally, several points of similarity between this growth and the Brown-Pearce tumor should be noted. The Brown-Pearce tumor also was composed of two cell types, an epithelioid and a "foamy" type. Metastases of both were present but, although both were used for transfer, only one type persisted. It is also of interest that the interim between the discovery of the initial growth and the occurrence of metastases was approximately the same in both cases. The close parallelism in behavior of the only two tumors of this type of which we have any knowledge may be of significance in respect to the rate of development of autonomy in the skin as compared to the breast or uterus of the rabbit.

SUMMARY

The course of a squamous cell carcinoma in the skin of a rabbit was followed from inception to death and clinical observations were supplemented by frequent biopsies and attempts to transfer the tumor. Histologically the primary tumor conformed in part to a type known in man as spindle cell epidermoid carcinoma, but the metastases were all squamous cell in character. The tumor was successfully transplanted and the histological character of the transplants varied according to locality.

REFERENCES

1. Brown, W. H., and Pearce, L. Studies Based on a Malignant Tumor of the Rabbit. I. The Spontaneous Tumor and Associated Abnormalities. J. Exper. Med., **37**:601-629. 1923.
2. Greene, H. S. N. Heterologous Transplantation of Mammalian Tumors. I and II. J. Exper. Med., **73**:461-486. 1941.
3. Greene, H. S. N. Heterologous Transplantation of a Human Fibrosarcoma. Cancer Research, **2**:649-654. 1942.
4. Greene, H. S. N. Familial Mammary Tumors in the Rabbit. IV. The Evolution of Autonomy in the Course of Tumor Development as Indicated by Transplantation Experiments. J. Exper. Med., **71**:305-324. 1940.
5. Greene, H. S. N. Familial Mammary Tumors in the Rabbit. III. Factors Concerned in Their Genesis and Development. J. Exper. Med., **70**:167-184. 1939.
6. Martin, H. E., and Stewart, F. W. Spindle-Cell Epidermoid Carcinoma. Am. J. Cancer, **24**:273-298. 1935.

Comparative Effects of Estrogen, Testosterone, and Progesterone on Benign Mammary Tumors of the Rat*

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The effects of estrogens and androgens on the growth of benign fibroadenoma have already been reported (6, 8). The present record deals with the effects on similar tumors of progesterone, and compares the effects of estrogen, androgen, and progesterone injected singly or in combination.[1] An attempt has been made to duplicate on tumors the effect of progesterone acting synergistically with estrogen on either the duct or alveolar system, or both, of the mammary gland in early pregnancy.

EXPERIMENTAL

Intact and castrated male and female rats with spontaneous benign tumors or auto- or homotransplants were injected with progesterone. Some were treated with estrogen previously, some simultaneously, and some received only progesterone. Two pregnant rats with benign tumors were also given progesterone. The total dosage varied between 9 and 18 mgm. of progesterone and 1 to 2.5 mgm. of estrogen. Sixty rats were used, including 4 with spontaneous tumors and 2 pregnant ones.

Before injections of estrogen or progesterone were begun 4 Wistar rats of our laboratory strain and bearing spontaneous tumors were inoculated with autotransplants. This gave an opportunity to study 3 to 4 growing or receding tumors of similar morphology in one rat. In these 4 rats there were available 12 autotransplants of the first generation, 6 of the second generation, and 2 of the third. In this way the aging of the tumor was slowed, and observation could be carried out on small tumors instead of on bulky ones (9).

Autotransplants were put into 4 and homotransplants into 60 Wistar animals, divided into the following series:

(a) Four females with spontaneous tumors.
(b) Thirty-eight normal females (2 pregnancies).
(c) Six castrated females and 6 normal males.
(d) Twelve castrated males.

Group I.—Four white female Wistar rats each with one spontaneous mammary fibroadenoma. These animals had littered from 3 to 5 times, and in two (R 2479 and R 2489) the tumors appeared during the last pregnancy. In one of these (R 2479) autotransplants were introduced into both axillae and the right groin, the primary tumor being left *in situ* in the left groin. Two mgm. of dimenformon (α-estradiol) were injected in divided doses during 3 weeks. The primary fibroadenoma and the autotransplants grew rapidly in the next 2 weeks, becoming adenomas, and the nipples became prominent. At the end of this time, 18 mgm. of progesterone were injected subcutaneously in divided doses during the next 9 weeks. Interval biopsies showed a gradual recession of the tumors and a shrinkage of their ducts and glands, with increasing fibrosis in the stroma. An additional 1 mgm. of estradiol restored their growth rate and they now showed microscopically a decided duct hyperplasia. At this stage 15 mgm. of testosterone propionate, injected subcutaneously in divided doses during 8 weeks, again caused recession with the microscopic appearance of epithelial necrosis. The ovaries and thyroid were involuted, the adrenals and pituitary decidedly enlarged. A hydrosalpinx of the right tube was present.

In the second rat (R 2489) with a spontaneous tumor and autotransplants 13 mgm. of progesterone were injected in 3 weeks. The primary tumor gradually diminished in size, grew harder, and showed involution of the glands and ducts (Figs. 1 and 2). The autotransplants did not grow. Injection of 1 mgm. of α-estradiol, after a second autotransplantation, stimulated the growth of the primary tumor and autotransplants after 6 weeks (Fig. 3). When the growth was

* Presented at the Endocrine-Cancer Conference, Atlantic City, June 5, 1942.

[1] Dimenformon benzoate was generously supplied by Roche-Organon, Inc., Nutley, N. J.; progesterone and testosterone by Ciba Pharmaceutical Products, Inc., Summit, N. J., Roche-Organon, Inc., Nutley, N. J., and Schering Corporation, Bloomfield, N. J.

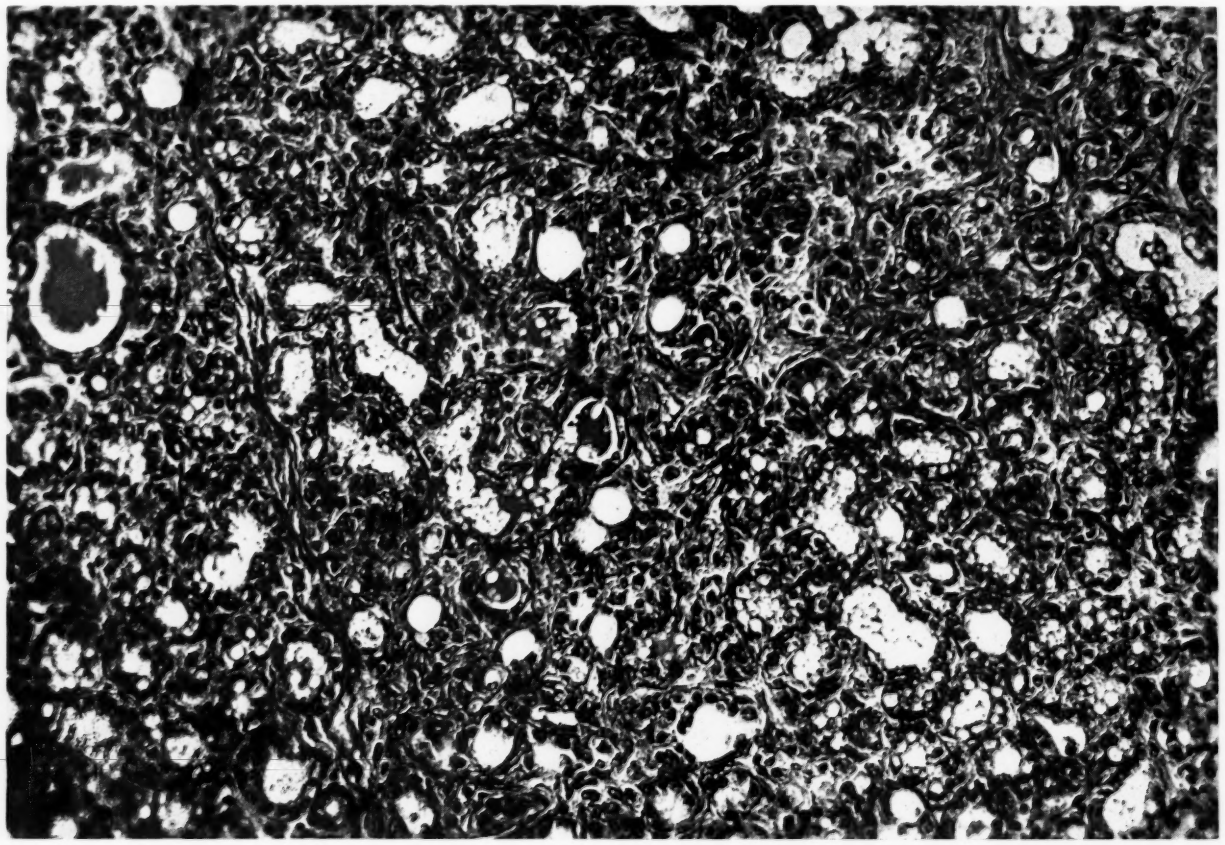

FIG. 1.—Spontaneous mammary adenofibroma in an untreated female white rat (R 2489). Mag. about × 300.

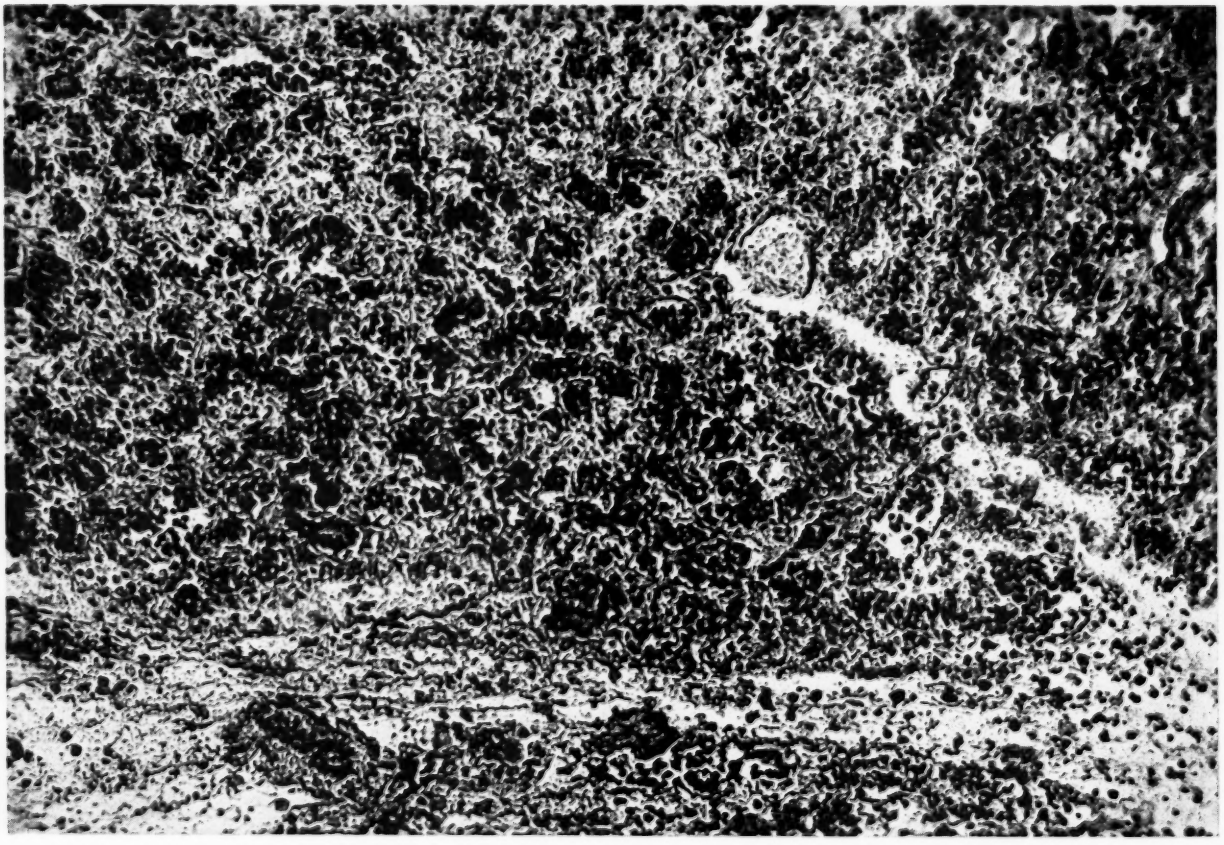

FIG. 2.—The same tumor after the subcutaneous injection of 18 mgm. of progesterone. Mag. about × 300.

established, the injection of 10 mgm. of testosterone propionate again caused a recession of the tumors. Alternating the injections of progesterone and testosterone with estradiol produced further inhibition or stimulation of the primary tumor and the autotransplants.

In 2 rats, each with a spontaneous and autotransplanted fibroadenoma, receiving only progesterone, the tumor remained stationary but involution of the glandular fraction was apparent. Autotransplants did not grow.

Group III.—In 6 castrated females and 6 normal males the injection of progesterone was ineffective. The few growing tumors were fibromatous, and the takes were reduced to 16 per cent. It had been previously reported that the administration of estrogen to female castrates restored the tumor takes to 54 per cent, and that the transplanted tumors remained fibroadenomas (7).

Group IV.—In 6 castrated males the transplanted tumors likewise became fibromas after the injection of 13 mgm. of progesterone. The fibroadenomas

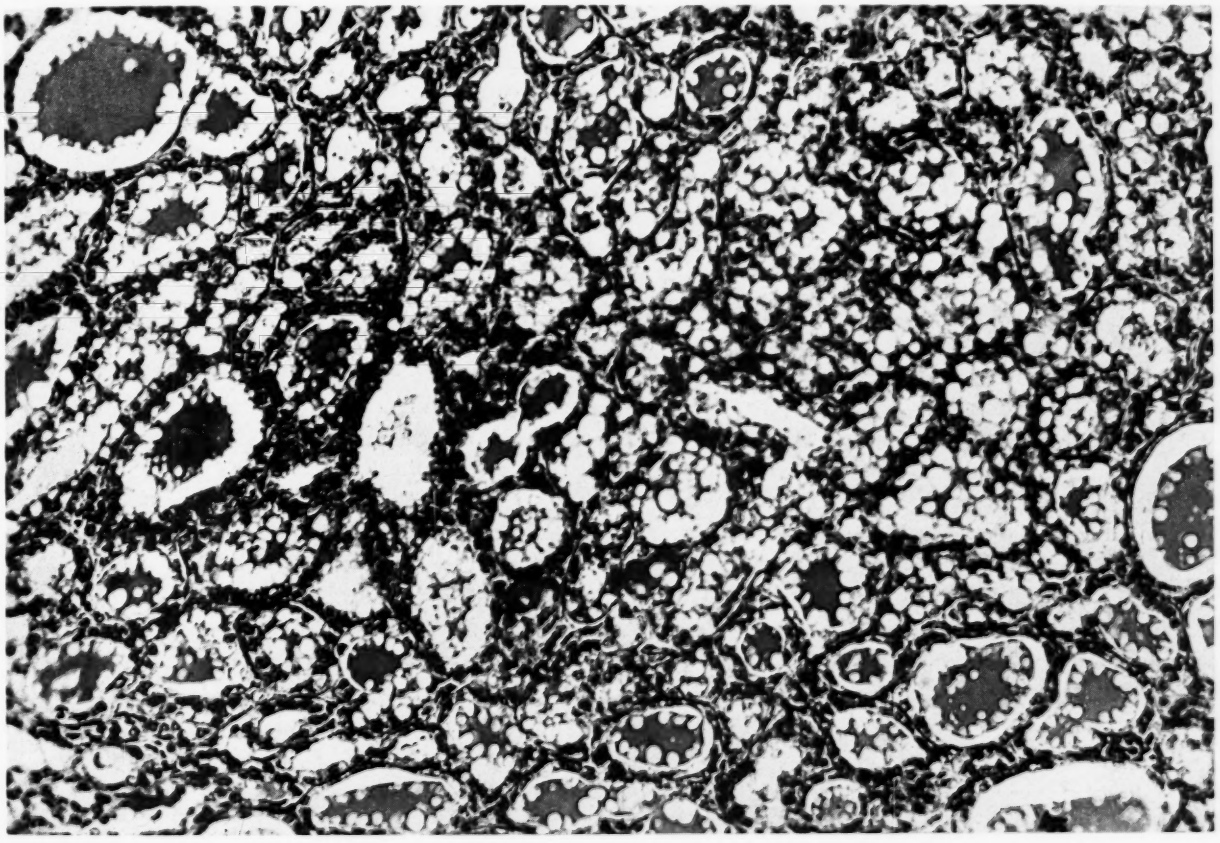

FIG. 3.—The same tumor as Figs. 1 and 2 after the subcutaneous injection of 2.5 mgm. of α-estradiol. Mag. about × 300.

Group II.—(a) Eighteen females with homotransplanted fibroadenoma were injected with 2.5 mgm. of estradiol followed by 18 mgm. of progesterone. In this group the effect of the estrogen on the benign tumors was evident, but there appeared to be no enhancing effect from the progesterone. The percentage of takes approximated the normal (66 per cent).

(b) Twenty females with homotransplanted fibroadenoma received 13 mgm. of progesterone each. In this group, the growth rate of the tumors was reduced (22 per cent) and most of them became cellular fibromas except in the 2 pregnant females, where the implanted fibroadenomas remained unchanged.

usually became more glandular in untreated castrated males (6, 7) and frequently formed adenomas when the male castrate was given estrogen.

In groups IIb, III, and IV the tumors were much smaller and harder, and the latent period was prolonged beyond 6 weeks. The action of progesterone on these growths resembled that of testosterone propionate on similar ones, and appeared to be indifferent or antagonistic to the action of estrogens. It required considerably larger doses of progesterone, as it did of testosterone, to overcome the action of estrogen.

Involution of the tumors, with regression and necrosis of the glands and ducts and an increased ten-

dency toward fibrosis, resulted when from 10 to 15 mgm. of testosterone propionate were administered in addition to progesterone. The number of takes in normal females was then reduced to 8.3 per cent. In 12 Wistar females inoculated with spontaneous fibroadenoma from a Wistar rat that had received progesterone, no growths resulted

DISCUSSION

The observations recorded here conform to some reports in the literature and are at variance with others.

Thus Corner (2) reported that progestin, injected into spayed rabbits, does not induce proliferation of the mammary gland. Turner and Frank (20) confirmed these findings in castrated male rabbits after estrogen followed by progestin. Turner and Schultze (22) found that stimulation of the mammary lobules did not occur in immature male and female castrated rats after luteal extracts were administered. Selye, Brown, and Collip (18) noted no effect in castrated females. Turner and Gomez (21) found progestin ineffective in stimulating growth. Nelson (14) modified his conclusion, at first (17) reporting stimulation and later (15) obtaining negative results from purified progestin. Lipschütz and Vargas (11, 12) described the prevention of experimentally produced uterine and extrauterine fibroids by the injection of testosterone and progesterone in guinea pigs. Nelson and Pfiffner (16) elicited notable hypertrophy of the mammary glands of immature castrated guinea pigs and male rabbits by injection of corpus luteum extract. Hartman and Speert (5) produced development of the mammary gland in castrated female monkeys after large doses (150 to 500 mgm.) of progesterone. Gardner and Hill (3) found that progestin stimulated duct growth in male and female mice. Astwood and Geschickter (1, 4) noted on effect on the mammary glands of rats after the administration of progesterone. Korenchevsky and Hall (10) found progesterone to inhibit uterine epithelial hyperplasia in estrinized rats.

Lyons and McGinty (13) reported that large doses of progesterone with small doses of estrone, given to immature male rabbits, inhibited mammary growth. An interesting observation by Smith and Werthessen pointed out the low pregnandiol values in chorioepithelioma (19).

The majority of investigators report a negative or inhibiting action of progesterone alone on normal mammary development, and stress the necessity of previous estrogen administration for complete physiologic development. No progestational effect has been noted on benign mammary fibroadenoma of the rat. In large doses, administered over a long period, progesterone seems to have an inhibitory effect on the glandular component of these tumors. This action resembles one noted after the administration of testosterone to rats with similar tumors.

CONCLUSIONS

1. Progesterone inhibited the growth of the adenomatous portion of spontaneous rat mammary fibroadenoma. Shrinkage was followed by fibrosis.
2. Progesterone reduced the percentage of takes of auto- and homotransplants (66 to 22 per cent).
3. Eighteen mgm. of progesterone did not interfere with the stimulating effect of 2.5 mgm. of estrogen. Eighteen mgm. of progesterone inhibited the stimulating effect of 1 mgm. of estrogen.
4. Progesterone alone did not affect fibromas growing in castrated females or normal males.
5. Progesterone alone inhibited growth of the glandular portion of adenofibromas in castrated males.
6. Progesterone and testosterone in combination were effective in reducing the percentage of takes from 66.6 to 8.3 per cent, and in inhibiting the growth of the glandular fraction of the tumor.
7. Large doses of progesterone or testosterone were necessary to neutralize the stimulating effect of estrogen on growing fibroadenoma.
8. Rat fibroma, myxoma, and sarcoma were not inhibited by progesterone.
9. Progesterone did not hinder the growth of fibroadenomas in pregnant rats, no doubt because of the high estrogen content of the organism.
10. The action of progesterone on benign mammary fibroadenomas was unlike the progestational effect on the uterus and normal mammary gland.

REFERENCES

1. Astwood, E. B., and Geschickter, C. F. Changes in the Mammary Gland of the Rat, Produced by Various Glandular Preparations. Arch. Surg., **36**:672-697. 1938.
2. Corner, G. W. The Hormonal Control of Lactation. I. Non-Effect of the Corpus Luteum. Am. J. Physiol., **95**:43-55. 1930.
3. Gardner, W. U., and Hill, R. T. Effect of Progestin upon the Mammary Glands of the Mouse. Proc. Soc. Exper. Biol. & Med., **34**:718-720. 1936.
4. Geschickter, C. F., and Astwood, E. B. The Relation of Oestrin and Other Hormones to Tumor Formation in the Breast. Cancer Probl., Symposium, Science, suppl., **85**:76-85. 1937.
5. Hartman, C. G., and Speert, H. Action of Progesterone on the Genital Organs of the Unprimed Rhesus Monkey. Endocrinology, **29**:639-648. 1941.
6. Heiman, J. The Study of Benign Neoplasms of the Rat's Breast. Am. J. Cancer, **22**:497-524. 1934.
7. Heiman, J. Growth of Transplanted Mammary Fibroadenoma in Castrated Rats Injected with Hormones. Am. J. Cancer, **39**:172-184. 1940.
8. Heiman, J. The Effect of Androgens and Estrogens on Spontaneous Benign Mammary Tumors in the Rat. Am. J. Cancer, **40**:343-354. 1940.

9. Heiman, J., and Krehbiel, O. F. The Influence of Hormones on Breast Hyperplasia and Tumor Growths in White Rats. Am. J. Cancer, **27**:450-473. 1936.
10. Korenchevsky, V., and Hall, K. The Effect of Progesterone on the Metaplasia of the Uterine Epithelium of Rats Injected with Oestrogens. J. Obst. & Gynaec. Brit. Emp., **45**:22-29. 1938.
11. Lipshütz, A., Murillo, R., and Vargas, L., Jr. Antitumorigenic Action of Progesterone. Lancet, **2**:420-421. 1939.
12. Lipschütz, A., and Vargas, L., Jr. Prevention of Experimental Uterine and Extrauterine Fibroids by Testosterone and Progesterone. Endocrinology, **28**:669-675. 1941.
13. Lyons, W. R., and McGinty, D. A. Effects of Estrone and Progesterone on Male Rabbit Mammary Glands. I. Varying Doses of Progesterone. Proc. Soc. Exper. Biol. & Med., **48**:83-86. 1941.
14. Nelson, W. O. Endocrine Control of the Mammary Gland. Physiol. Rev., **16**:488-526. 1936.
15. Nelson, W. O. Studies on the Physiology of Lactation. VI. The Endocrine Influences Concerned in the Development and Function of the Mammary Gland in the Guinea Pig. Am. J. Anat., **60**:341-359. 1937.
16. Nelson, W. O., and Pfiffner, J. J. An Experimental Study of the Factors Concerned in Mammary Growth in Milk Secretion. Proc. Soc. Exper. Biol. & Med., **28**:1-2. 1930.
17. Nelson, W. O., and Pfiffner, J. J. Studies on the Physiology of Lactation. I. The Relation of Lactation to the Ovarian and Hypophyseal Hormones. Anat. Rec., **51**:51. 1931.
18. Selye, H., Browne, J. S. L., and Collip, J. B. Effect of Combined Administration of Oestrone and Progesterone in Adult Ovariectomized Rats. Proc. Soc. Exper. Biol. & Med., **34**:198-200. 1936.
19. Smith, J. T., and Werthessen, N. T. Chorioepithelioma Associated with Tubal Pregnancy and Pulmonary Metastases. High Gonadotropin and Low Pregnanediol Values. Am. J. Obst. & Gynec., **41**:153-156. 1941.
20. Turner, C. W., and Frank, A. H. The Relation between the Estrus-Producing Hormone and a Corpus Luteum Extract on the Growth of the Mammary Gland. Science, **73**:295-296. 1931.
21. Turner, C. W., and Gomez, E. T. The Experimental Development of the Mammary Gland. I. The Male and the Female Albino Mouse. II. The Male and the Female Guinea Pig. Missouri Agr. Exper. Sta. Research Bull., No. 206. 1934.
22. Turner, C. W., and Schultze, A. B. A Study of the Causes of the Normal Development of the Mammary Glands of the Albino Rat. Missouri Agr. Exper. Sta. Research Bull., No. 157. 1931.

Abstracts

Reports of Experimental Research

Carcinogenic Compounds

ANDERVONT, H. B., and SHEAR, M. J. [Nat. Cancer Inst., Bethesda, Md.] PRODUCTION OF TUMORS IN MICE FOLLOWING THE REMOVAL OF METHYLCHOLANTHRENE-CHOLESTEROL PELLETS. J. Nat. Cancer Inst., 2:333-334. 1942.

Cholesterol pellets containing 5% methylcholanthrene were implanted in C3H mice and subsequently removed 2 to 12 weeks after implantation. Two weeks' exposure produced 23% tumors within 22 weeks, whereas 6 weeks' exposure produced 88% tumors within 20 weeks. When the pellets were left in place, the tumor incidence was 100% and the average time for the appearance of the tumors was 14 weeks.—A. C.

COOPER, Z. K., and RELLER, H. C. [Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] MITOTIC FREQUENCY IN METHYLCHOLANTHRENE EPIDERMAL CARCINOGENESIS IN MICE. J. Nat. Cancer Inst., 2:335-344. 1942.

Both ears of each of 52 mice were painted twice weekly with a 0.6% methylcholanthrene solution in benzene, and mitotic counts were made on total mounts of the epidermis of the ears of 3 mice each week. The results are based on counts of 15,000 nuclei in each ear. From this experiment, it would seem that methylcholanthrene acts first as a stimulant to cell division, this being followed by a period in which mitotic figures are less abundant. When this period of apparently reduced activity is passed and carcinoma is about to develop, a pronounced increase in mitotic activity again occurs.—A. C.

Biochemistry and Nutrition

GREENSTEIN, J. P. [Nat. Cancer Inst., Bethesda, Md.] A METHOD OF EVALUATING THYMONUCLEODEPOLYMERASE ACTIVITY IN NORMAL AND TUMOR TISSUES. J. Nat. Cancer Inst., 2:357-359. 1942.

A method is described whereby the thymonucleodepolymerase activity of various tissues can be defined in absolute numerical terms.—A. C.

GREENSTEIN, J. P., and ANDERVONT, H. B. [Nat. Cancer Inst., Bethesda, Md.] THE LIVER CATALASE ACTIVITY OF TUMOR-BEARING MICE AND THE EFFECT OF SPONTANEOUS REGRESSION AND OF REMOVAL OF CERTAIN TUMORS. J. Nat. Cancer Inst., 2:345-355. 1942.

The liver catalase activity of mice bearing the following tumors was found to be much lower than normal: in *C3H* mice, transplanted adenocarcinoma, methylcholanthrene-induced sarcoma, endothelioma, and spontaneous mammary tumors; in *A* mice, transplanted lymphoma, pulmonary, and mammary tumors; in *C* mice, transplanted sarcoma 37 and hemangioma; in dilute brown mice, transplanted sarcoma 37, CR 180, and melanoma; in *I* mice, transplanted hepatoma and sarcoma 37; in *Y* mice, transplanted sarcoma 37. With few exceptions, the effect of the tumors on decreasing liver catalase activity was progressive with the growth of the tumors. After removal or regression of the tumors, the value of the liver catalase returned to the normal level. Mice of the C57 black strain provide an exception. In this case, the normal liver catalase activity was not appreciably affected by the growth or removal of a tumor.—A. C.

Review

SPENCER, R. R. [Nat. Cancer Inst., Bethesda, Md.] TUMOR IMMUNITY. J. Nat. Cancer Inst., 2:317-332. 1942.

The paper constitutes a critical review of the work on tumor immunity, especially that on record since the 1938 monograph of Clemmensen. A comprehensive list of the recent literature on the subject is appended. Recent observations are in agreement with the view that natural or induced resistance to transplanted tumor cells is controlled by normal immunological processes, in that the antigen (normal or tumor cells) must be closely related to the transplant (specificity) and the intensity of the response to immunization depends on the degree of foreignness between the tumor and the host. Changes in technic, such as the use of skin for immunization, may affect the results quantitatively, but the mechanisms involved appear to remain the same. Rare instances of successful heterotransplantation indicate that the resistance to the growth of foreign cells is not always effective, although induced resistance in the particular case of the Putnoky tumor conforms to normal immunological principles. The possibility that specific anti-cancer cell substances exist in the blood (Lumsden) has not been convincingly demonstrated. The role of the lymphocyte in tumor immunity is reviewed. The paper also includes a list of experiments in which attempts were made to affect the growth of transplanted tumor cells either by preliminary or concomitant treatment with cells, specific antigens, or antibodies. On present evidence, the prospect of a treatment of cancer by immunological means is not promising.—A. C.

Miscellaneous

LOEB, L., SUNTZEFF, V., BLUMENTHAL, H. T., and KIRTZ, M. M. [Washington Univ. Sch. of Med., St. Louis, Mo.] EFFECT OF WEIGHT ON THE DEVELOPMENT OF MAMMARY CARCINOMA IN VARIOUS STRAINS OF MICE. Arch. Path. 33:845-865. 1942.

Strains of mice differ in average weights at various ages and their weight curves are not parallel to the

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hereditary tendency of these strains to acquire mammary carcinoma. Within the different strains possessing a sufficient tendency to the development of mammary cancer, there exists a direct relation between the frequency of cancer and the average weight, the latter being greater in tumor-bearing mice. The greater the hereditary tendency to the development of mammary cancer, or the larger the dose of estrogen given, the more readily is the cancerous stage reached in mice belonging to a lower weight class.—H. G. W.

DISCUSSION ON GROWTH AND NEW GROWTH. Section of Surgery. Royal Society of Medicine, London. Proc. Roy. Soc. Med., 35:587-600. 1942.

A discussion in which H. Burrows, W. E. Gye, L. R. Broster, P. B. Medawar, F. G. Spear, and A. Haddow took part.—E. L. K.

Clinical and Pathological Reports

Radiation—Diagnosis and Therapy

ACKERMANN, A. J. [State Univ. and Crippled Children's Hosp., Oklahoma City, Okla.] PRIMARY TUMORS OF THE DIAPHRAGM ROENTGENOLOGICALLY CONSIDERED. Am. J. Roentgenol., 47:711-716. 1942.

After a brief review of the meagre literature, the author reports two cases of primary tumor of the diaphragm, confirmed in one instance by operation. Accurate localization of such tumors by radiological means requires fluoroscopic observation of the respiratory movements of the mass, and usually pneumoperitoneum and pneumothorax in order to rule out disease primary in the abdominal viscera or lungs.—C. E. D.

COLEY, B. L., and MILLER, L. E. [Memorial Hosp., New York, N. Y.] ATYPICAL GIANT CELL TUMOR. Am. J. Roentgenol., 47:541-548. 1942.

Seven cases of atypical giant cell tumor of bone are presented with illustrative roentgenograms. The tumors may be classed as atypical on the basis of clinical or pathological criteria or because of unusual location. The most important duty of the roentgenologist is to distinguish between benign and malignant lesions.—C. E. D.

GOIN, L. S. [Los Angeles, Calif.] THE MANAGEMENT OF PATIENTS RECEIVING RADIATION THERAPY FOR CANCER. Radiology, 38:513-515. 1942.

Every effort should be made to improve the general condition of a patient before subjecting him to massive radiation therapy. Prior to and during treatment the patient's mental as well as his physical state deserves attention. Radiation sickness may be combatted with liver extract, vitamin B_1, and abundant glucose; menopausal symptoms may be controlled with estrogens. Unpleasant local affections, particularly dryness of the mouth, cystitis, and proctitis, should receive palliative treatment.—C. E. D.

HODGES, F. M., SNEAD, L. O., and BERGER, R. A. [Richmond, Va.] A STELLATE IMPRESSION IN THE CARDIAC END OF THE STOMACH SIMULATING TUMOR. Am. J. Roentgenol., 47:578-582. 1942.

A peculiar shadow in the cardia of the stomach, suggesting a filling defect, was seen in 47 of 1,500 roentgenologic examinations of the stomach. In several cases tumor was suspected. The shadow consists of an opaque stellate center surrounded by a halo of radiolucence 1 to 2 cm. or more in diameter. It is located exactly at the esophageal-gastric juncture. This sign is produced by barium in the stellate ostium of the esophagus and a slight protrusion of the esophageal mucosa into the stomach. It can be distinguished from carcinoma by the free passage of barium through the apparent filling defect.—C. E. D.

KENNEY, J. M., MARINELLI, L. D., and CRAVER, L. F. [Memorial Hosp., New York, N. Y.] THE TREATMENT OF LYMPHOSARCOMA WITH RADIOACTIVE PHOSPHORUS. A PRELIMINARY REPORT. Am. J. Roentgenol., 47:217-226. 1942.

Patients with lymphosarcoma were given small tracer doses of radioactive phosphorus (P^{32}). Subsequent biopsies of lymph nodes at intervals of 1 to 31 days showed selective concentration of radioactivity in the nodes. Eighteen patients were therefore given therapeutic doses of P^{32} ranging from 145 to 380 μc. per kg. of body weight. Seventeen patients are still living, the longest period since the beginning of treatment being 8 months. Five patients have had complete regression of the enlarged lymph nodes. Nine case histories are presented. Some bone marrow damage is to be expected and frequent blood counts are advised. If the white count falls below 3,500, the platelets below 150,000, or the red count drops more than 15%, therapy should be discontinued. In spite of the clinical improvement in many of these patients, 6 lymph node biopsies made during treatment showed no histological changes attributable to radiation.—C. E. D.

KIRKLIN, B. R. [Mayo Clinic, Rochester, Minn.] THE MENISCUS-COMPLEX IN THE ROENTGENOLOGIC DIAGNOSIS OF ULCERATING CARCINOMA OF THE STOMACH. Am. J. Roentgenol., 47:571-577. 1942.

Ulcerating carcinomas of the stomach which do not penetrate the gastric wall are often difficult to diagnose roentgenologically. Manipulation of the barium-filled stomach will often result in filling the crater of such a tumor with barium while the elevated tumor border displaces barium, giving a fluoroscopic picture of a halo of radiolucence around an opaque center. When the tumor is on the lesser curvature the picture is modified by being viewed on edge. With the aid of excellent illustrations, Kirklin discusses the uses and abuses of this sign.—C. E. D.

LINGLEY, J. R. [Massachusetts Gen. Hosp., Boston, Mass.] THE SIGNIFICANCE OF PSAMMOMA CALCIFICATION IN THE ROENTGEN DIAGNOSIS OF PAPILLARY TUMORS OF THE OVARY. Am. J. Roentgenol., 47:563-570. 1942.

The diagnostic importance of calcification in lesions of the ovary has received little attention except in the case of dermoid cysts. Calcified psammoma bodies in an ovarian tumor can usually be detected roentgenologically by the hazy shadow of increased density cast by the innumerable minute bodies uniformly distributed through the tumor. Occasionally a much denser shadow is cast. Psammoma calcification in the ovary is practically limited to papillary cystadenomas and papillary cystadenocarci-

nomas. Five instances of psammoma calcification are presented, together with 6 roentgenograms. In 4 patients the findings justified a roentgenologic diagnosis of papillary cystadenocarcinoma of the ovary.—C. E. D.

POHLE, E. A., and BENSON, R. R. [Univ. of Wisconsin Med. Sch., Madison, Wis.] ROENTGEN THERAPY IN CANCER OF THE BREAST. AN ANALYSIS OF EXPERIENCES AT THE STATE OF WISCONSIN GENERAL HOSPITAL DURING THE LAST TWELVE YEARS. Radiology, 38:516-523. 1942.

Since 1930, postoperative roentgen irradiation has been routine in cases of cancer of the breast seen at the Wisconsin General Hospital. Recurrences, metastases, and advanced inoperable lesions are treated roentgenologically with good palliative results. The various technics in use are described. Since large doses are given, complications are frequent. Local skin reactions are treated with olive oil. Bed rest, small frequent meals, and a suppository of 2 grains of nembutal one hour before treatment are used in an attempt to forestall radiation sickness. Radiation fibrosis of the lungs was rarely encountered, but asymptomatic radiation osteitis of the ribs was common. The 5 year and 3 year survival of all patients was 38.1% and 48.8% respectively. Satisfactory palliation was obtained in 60% to 70% of patients with metastatic lesions.—C. E. D.

RENDICH, R. A., POPPEL, M. H., and COVE, A. M. [Kings County Hosp., Brooklyn, N. Y.] ROENTGEN DIAGNOSIS OF SPACE-OCCUPYING LESIONS OF THE HEAD OF THE PANCREAS. Radiology, 38:47-52. 1942.

In addition to carcinoma of the head of the pancreas, many other space-occupying lesions in this region are capable of producing identical roentgenographic signs. Among these are: enlargement of retroperitoneal lymph nodes, pancreatitis, retroperitoneal tumor, cyst of the head of the pancreas, aneurysm of the anterior wall of the abdominal aorta, amyloidosis of the pancreas, and upward traction of the duodenum as in diaphragmatic hernia of the stomach. Seventeen roentgenograms are presented illustrating a number of these conditions.—C. E. D.

RICHARDS, G. E. [Toronto Gen. Hosp., Toronto, Canada] THE TREATMENT OF CANCER OF THE TONGUE. Am. J. Roentgenol., 47:191-205. 1942.

The problem of treatment of cancer of the tongue is considered in detail, including the classification of cases according to the size of the primary lesion, secondary involvement, location of the lesion, and histological type. Of the author's 167 patients 80% were males. Eighty-six per cent of the tumors were situated on the lateral margin or base of the tongue, and only 6% were less than 1.5 cm. in diameter when first seen. The histological diagnosis in 91% was epidermoid carcinoma and 50% of the patients had palpable lymph nodes. Twenty per cent of the patients had positive Wassermann reactions.

A technic of treatment is described by which large doses of external radiation generated at 400 kv. are supplemented by treatment at 200 kv. through an intraoral cone. A tumor dose in excess of 5,500 r generally controls lingual cancer except for lesions on the dorsum of the tongue.

Of a total of 119 patients treated since 1929, 37% were living and free from disease after 3 years and 27% after 5 years or more. The new method of radiation treatment was introduced in 1935 and has given 50% three year survivals to date.—C. E. D.

SUTHERLAND, C. G. [Mayo Clinic, Rochester, Minn.] THE ROENTGENOLOGIC DIAGNOSIS OF TUMORS OF BONE. Am. J. Roentgenol., 47:534-540. 1942.

The various forms of benign and malignant bone tumor are discussed and the roentgenologic characteristics which aid in differential diagnosis are presented.—C. E. D.

TYLER, A. F. [Omaha, Neb.] IRRADIATION THERAPY OF CANCER OF THE BREAST. Nebraska State M. J., 26: 424-428. 1941.

A discussion of the accepted methods.—M. J. E.

UHLMANN, E., and GROSSMAN, A. [Michael Reese Hosp., Chicago, Ill.] THE USE OF RADON OINTMENT AS A MEANS OF DIFFERENTIATION BETWEEN RADIONECROSIS AND RECURRENT CARCINOMA. Am. J. Roentgenol., 47:620-623. 1942.

It is stated that radon ointment, prepared by taking up small amounts of radium emanation in vaseline, is so effective in promoting the healing of radiation ulcers that an ulcer in an irradiated region which does not heal under this treatment should be suspected of being recurrent carcinoma. If the ulcer does heal the diagnosis of cancer may be discarded.—C. E. D.

Gastrointestinal Tract

CAVE, H. C. [Roosevelt Hosp., New York, N. Y.] CANCER OF THE RECTUM. J. M. Soc. New Jersey, 38:468-472. 1941.

The author gives the results of operative intervention in carcinoma of the rectum. Despite radical resection the percentage of cured patients is small. Of 91 patients observed, 57 had tumors considered suitable for a radical intervention, and 12 died postoperatively. Of those surviving, 15 lived less than 1 year, 9 lived more than 1 year, 7 more than 2 years, 4 more than 3 years, 5 more than 4 years, and 5 for 5 years or longer.—M. J.E.

RITVO, M., and HEWES, F. L. [Pondville Hosp., Wrentham, Mass.] CLINICAL AND ROENTGEN MANIFESTATIONS OF CARCINOMA OF THE DUODENUM. Radiology, 38:7-13. 1942.

The clinical course of the disease and the autopsy findings in 3 cases of carcinoma of the duodenum are reported. Two roentgenograms are reproduced, and diagnosis and treatment are discussed.—C. E. D.

TEMPLETON, F. E., and BOYER, R. C. [Univ. of Chicago, Chicago, Ill.] THE DIAGNOSIS OF GASTRIC CANCER; AN ANALYSIS OF THE GASTROSCOPIC AND ROENTGENOLOGIC FINDINGS. Am. J. Roentgenol., 47:262-274. 1942.

A valuable analysis is presented of the relative merits of roentgenologic and gastroscopic diagnosis in benign and malignant lesions of the stomach. From a total of 1,072 cases examined by both methods, 189 examples of ulcerating polypoid and infiltrating lesions together with some instances of gastritis were selected for study. The nature of the lesion was confirmed histologically in 74 cases. In the majority of instances the gastroscopist and roentgenologist reached the same diagnosis at the initial examination. Some lesions, by virtue of location or morphology, were visible by only one method. Occasionally lesions were correctly located by both methods but different diagnoses reached. In these lesions the gastroscopist was no more and no less likely to be correct than the roentgenologist. The authors conclude that a combination of gastroscopic and roentgenologic examination will result in a higher percentage of correct diagnoses than either method used alone.—C. E. D.